

What kind of drugs should we develop

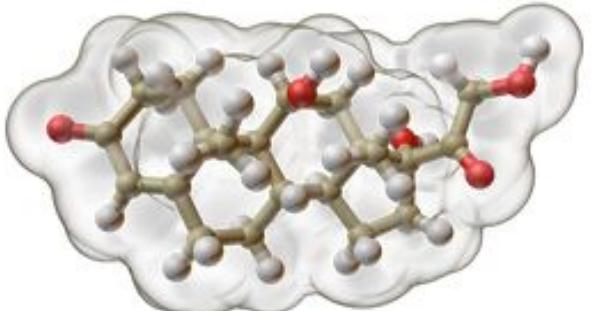
*Mathematical and Computational Biology in Drug Discovery
(MCBDD) Module III*

*Dr. Jitao David Zhang
April 2021*

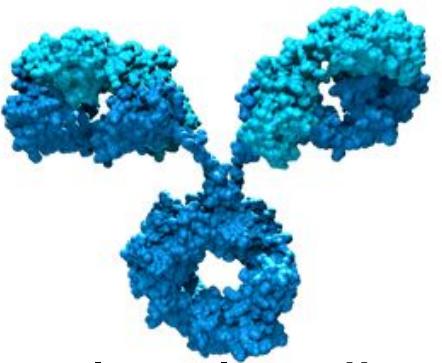
Overview

- Essentials of modalities
 - Small molecules: classical, protein degrader, RNA modulator
 - Large molecules: classical, DUTA-Fabs, protein design
 - Antisense oligonucleotides: siRNA, shRNA, ASO
 - Gene and cell therapy
- Three case studies:
 - Success stories:
 - [Small molecules] SMA (Evrysdi/Risdiplam and Nusinersen)
 - [Antisense] patisiran ([KEGG DRUG](#)) and givosiran ([DrugBank](#), [structure available at EMA](#))
 - [Offline read] mRNA vaccine (MIT Technology Review)
 - Turning failure into successes: [Multispecific drugs] Thalidomide, PROTAC, degraders
 - [Antibody] Cancer immunotherapy (CTLA4, PD1)
 - [Gene and Cell therapy] CAR-T
 - Challenges
 - [Antisense] HTT (Tominersen)
 - Difference between genetic and enzymatic inhibition

A zoo of modalities



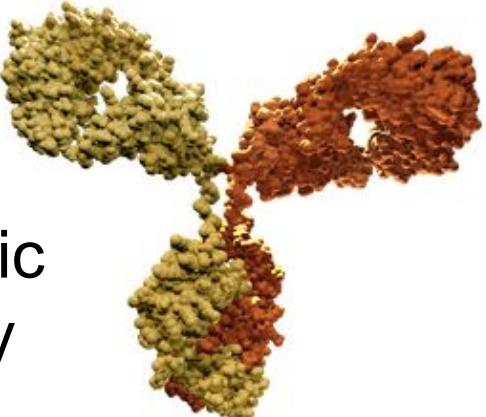
Small molecule



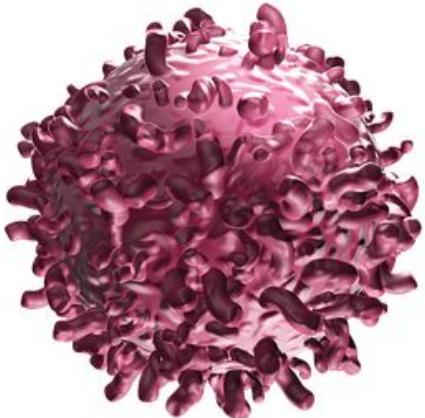
Monoclonal antibody



RNA inference



Bispecific
antibody



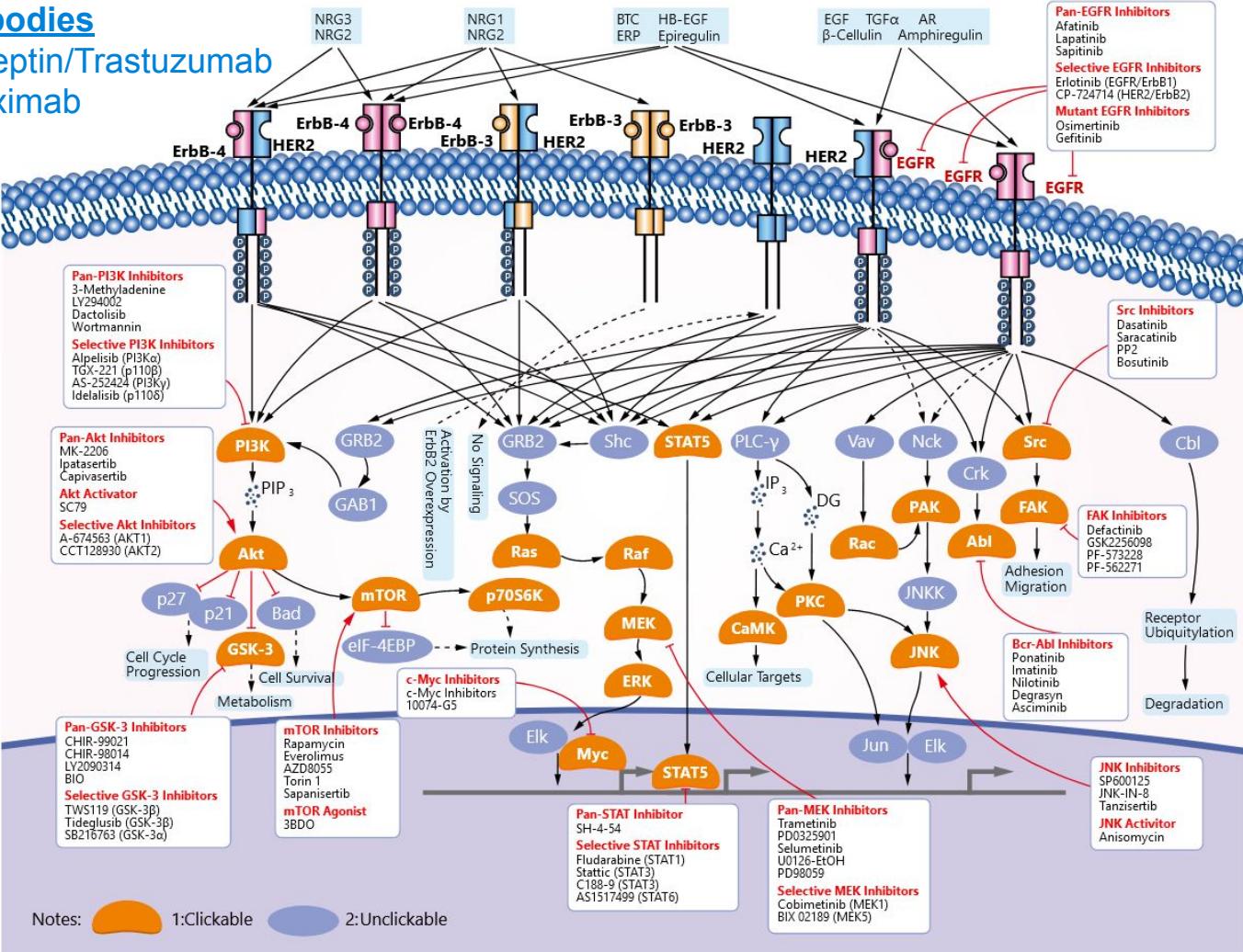
Chimeric
Antigen
Receptor
(CAR) T-cells

Multiple modalities can target the same biological process

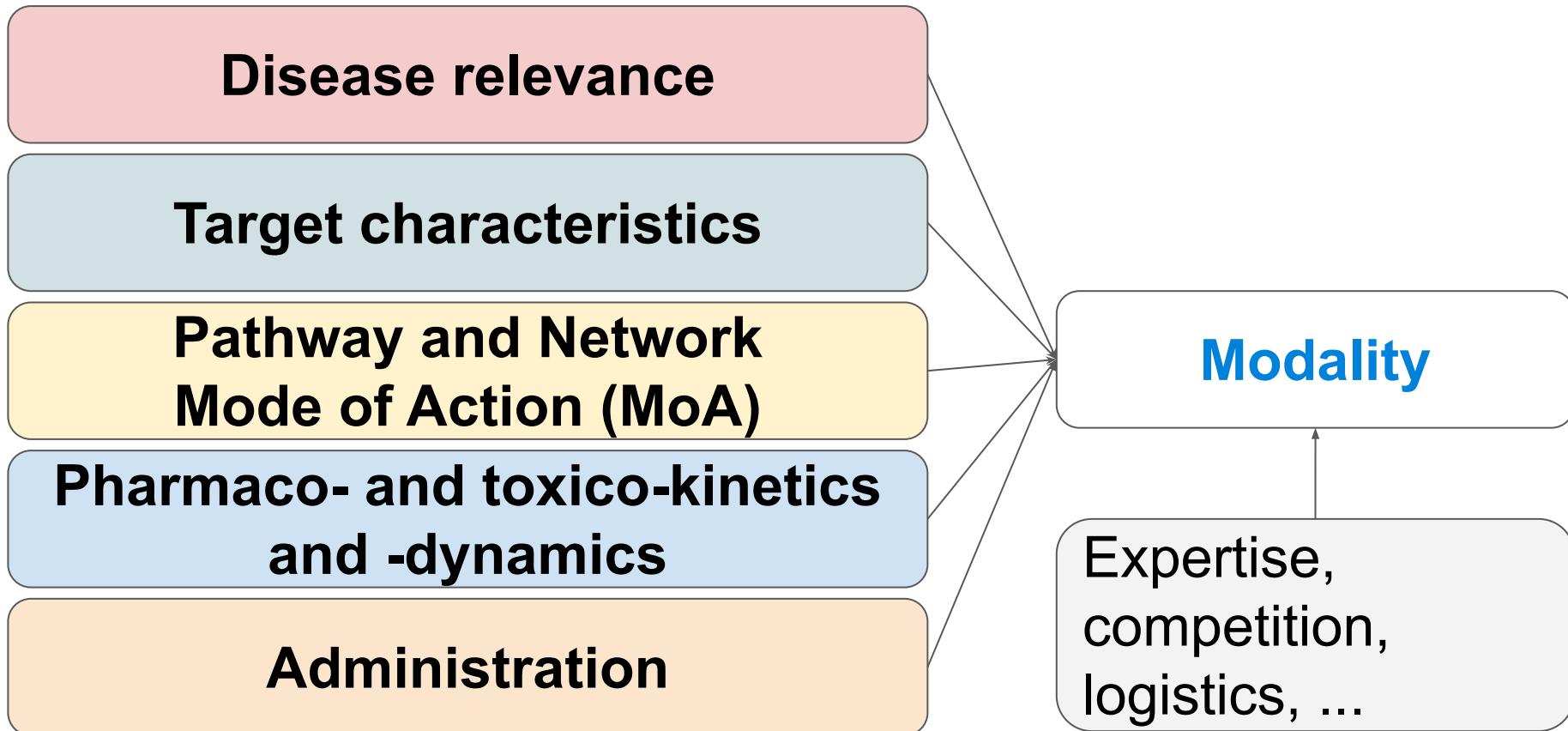
An example: the epidermal growth factor receptor (EGFR) pathway

Antibodies

Herceptin/Trastuzumab
Cetuximab



Criteria to choose a modality

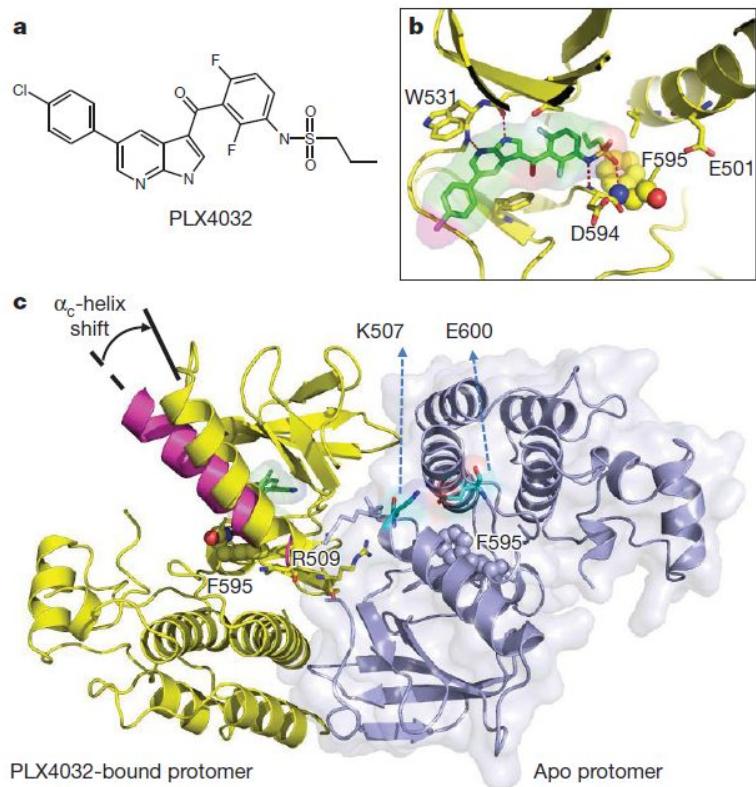


Characteristics of therapeutic modalities

Modality	Cause of disease at the protein level		Molecular target	Protein target localization			Delivery
	Reduction or loss of function	Excessive or detrimental function		Extracellular	Plasma membrane	Intracellular	
Small molecule	●	●	DNA → RNA → Protein	●	●	●	Oral Injection Inhaled
Protein replacement	○			○	○	○	○
Antibody		●		●	●		●
Oligonucleotide therapy	○	○	○	○	○	○	○
Cell and gene therapy*	●		●	●	●		●

Classical small molecules: an example from AMIDD

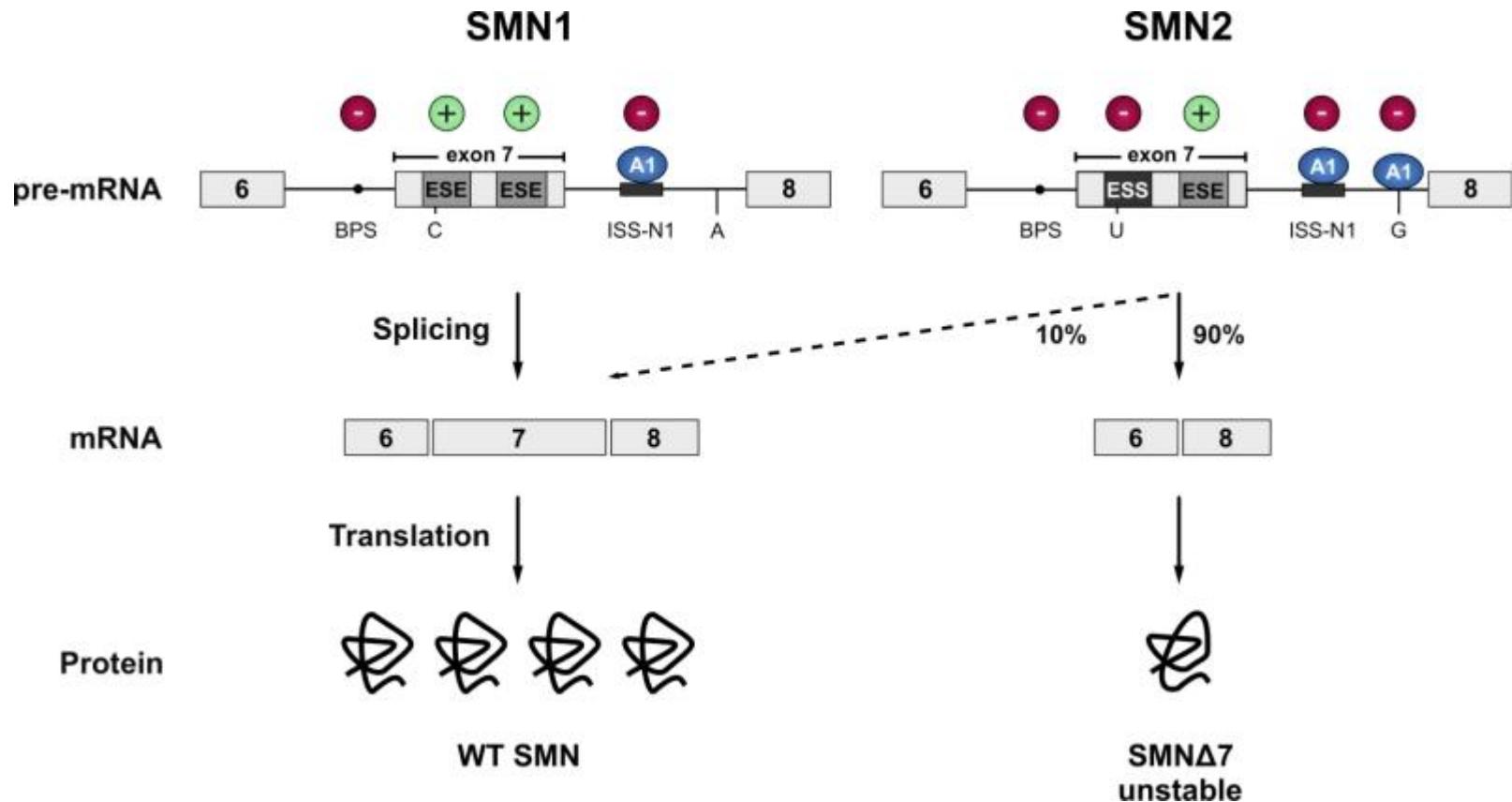
- Vemurafenib (Zelboraf, PLX4032)
V600E mutated BRAF inhibition
- Lock and key: an oversimplified yet powerful metaphor, first proposed by Emil Fischer



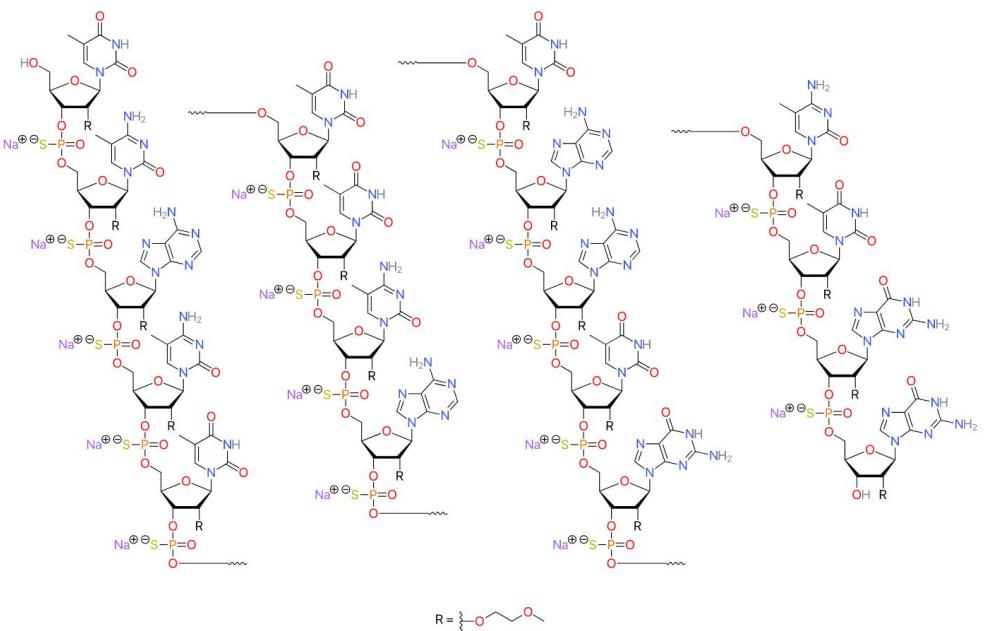
Facts about Spinal Muscular Atrophy (SMA)

- SMA is caused by a defect in a gene called *SMN1*. People with SMA have reduced levels of the SMN protein.
- When SMN protein levels are reduced, motor neurons are unable to send signals to the muscles, causing them to become smaller and weaker over time.
- Depending on the severity, or type of SMA, people with the disease will have difficulties moving, eating, and in some cases breathing, making them increasingly dependent on parents and caregivers.
- A short movie: <https://www.nejm.org/doi/full/10.1056/NEJMoa2009965>

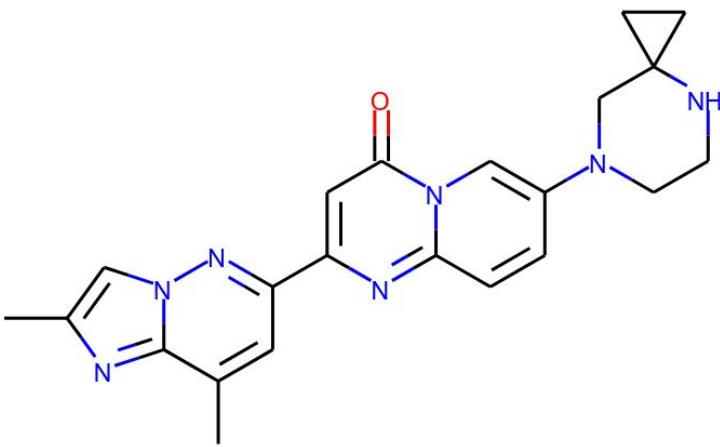
The molecular mechanism of SMA



Two Drugs, One Disease

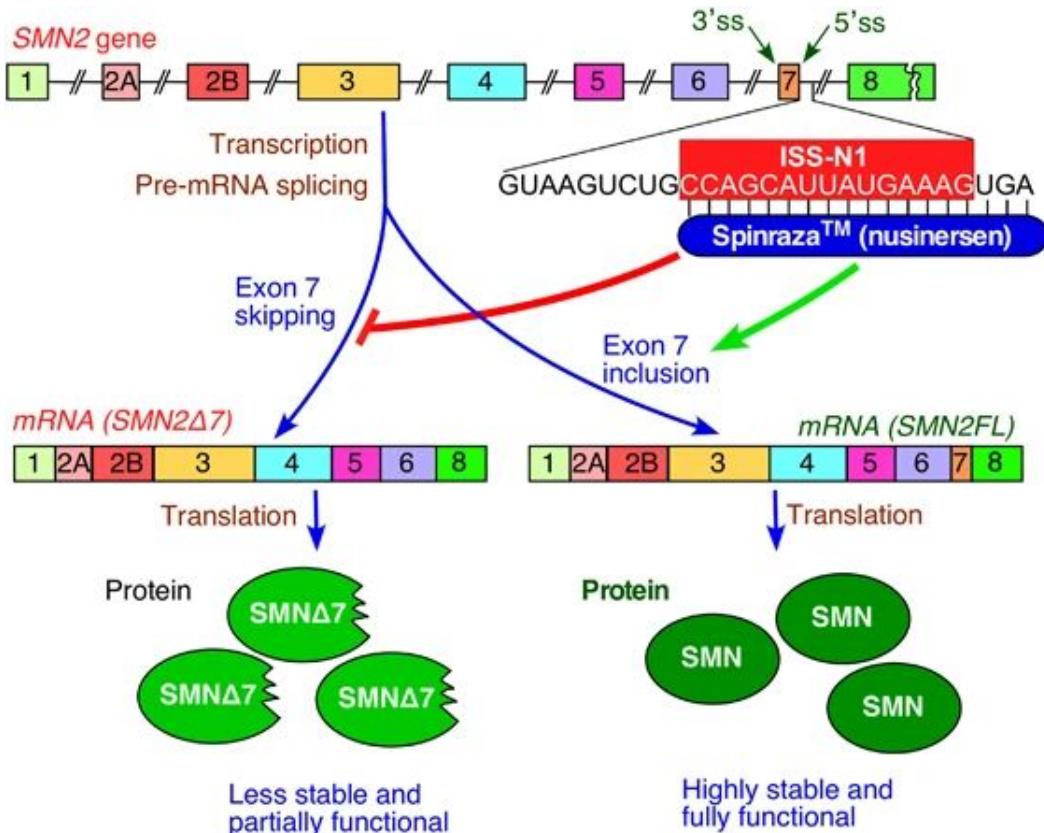


Nusinersen sodium/ Spinraza
[\(CHEMBL3833342\)](#)

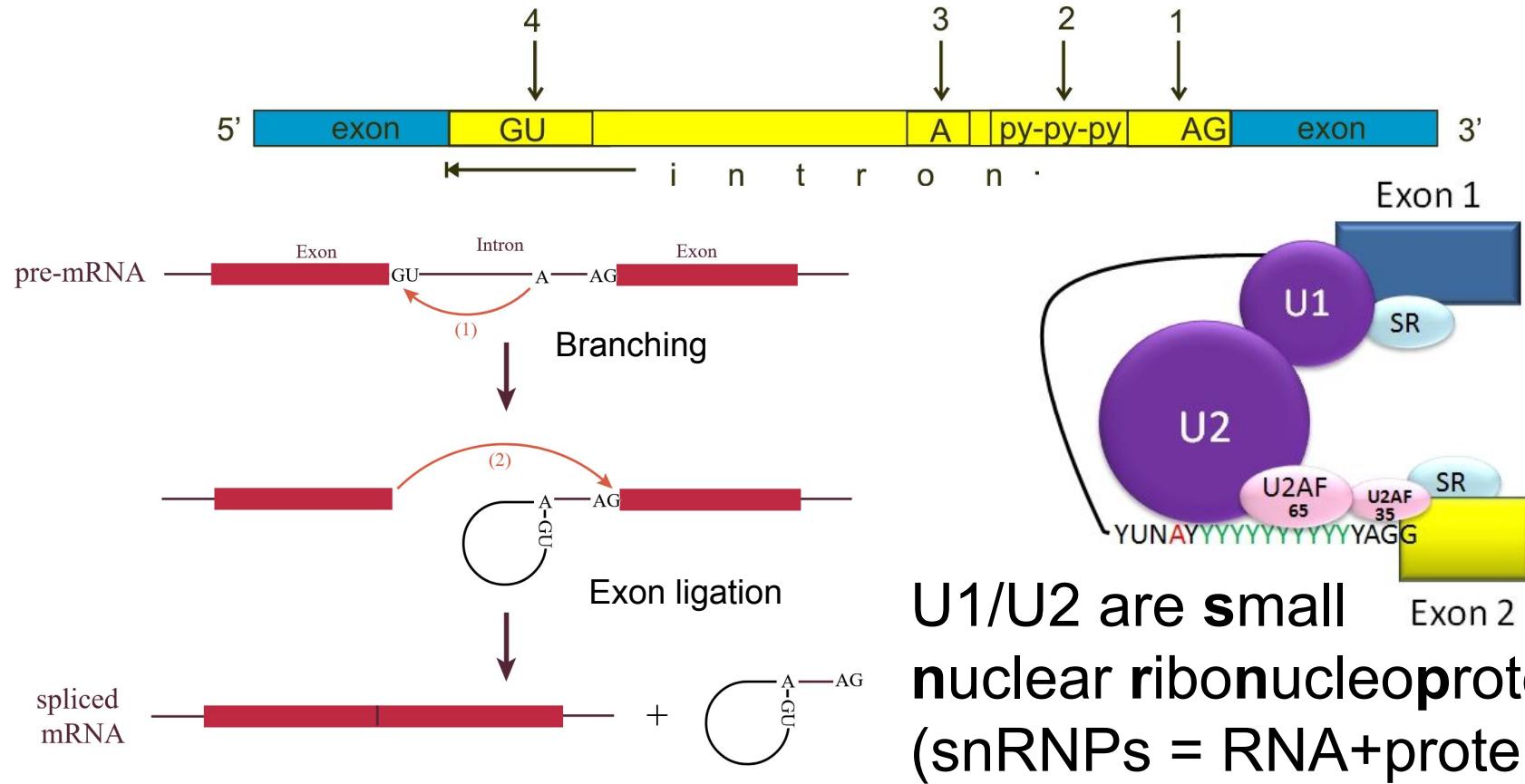


Risdiplam/ Evrysdi/
[\(CHEMBL4297528\)](#)

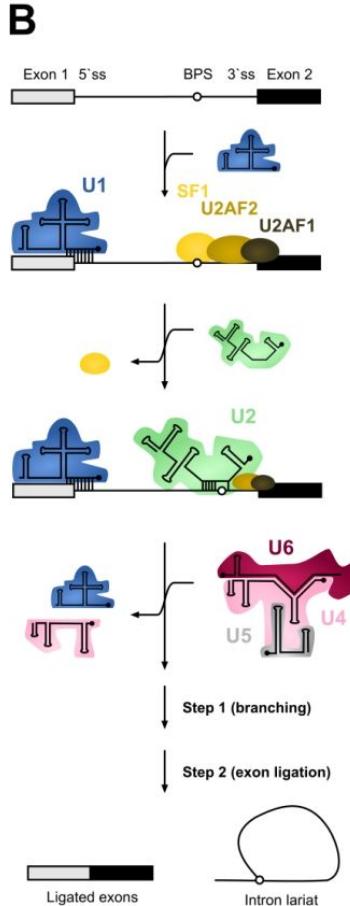
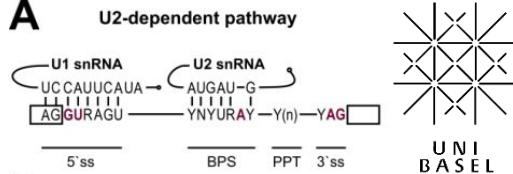
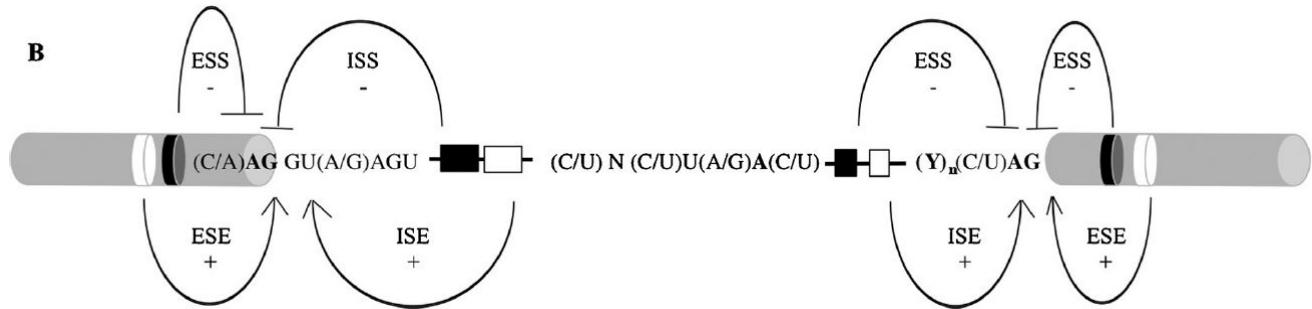
How Spinraza (nusinersen) works



Spliceosome: the splicing machinery



Splicing in action and under regulation

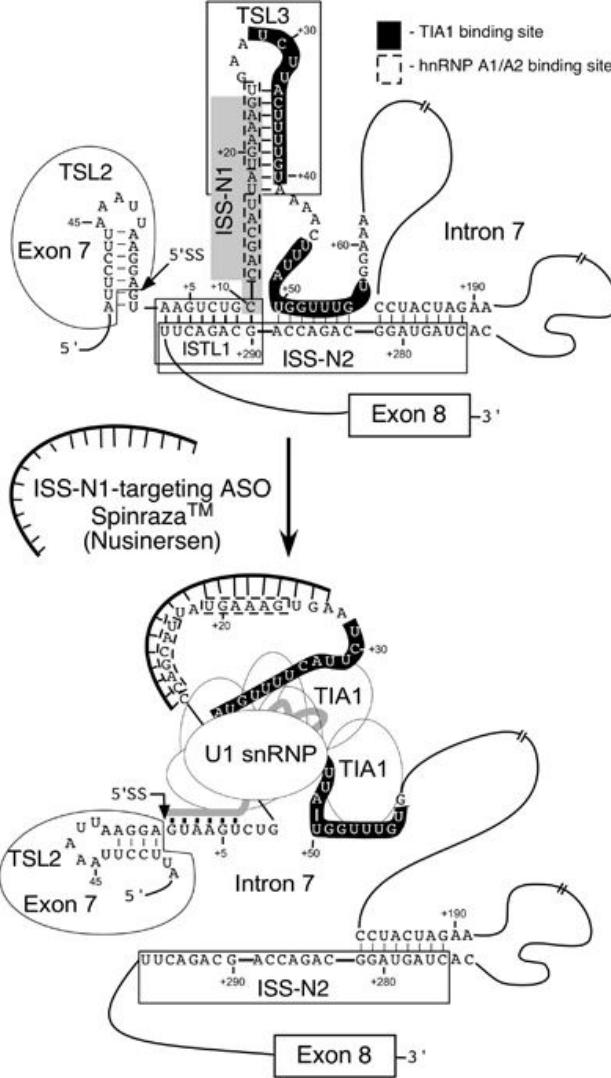


BPS=branch point sequence; PPT=polypyrimidine tract (C/U);

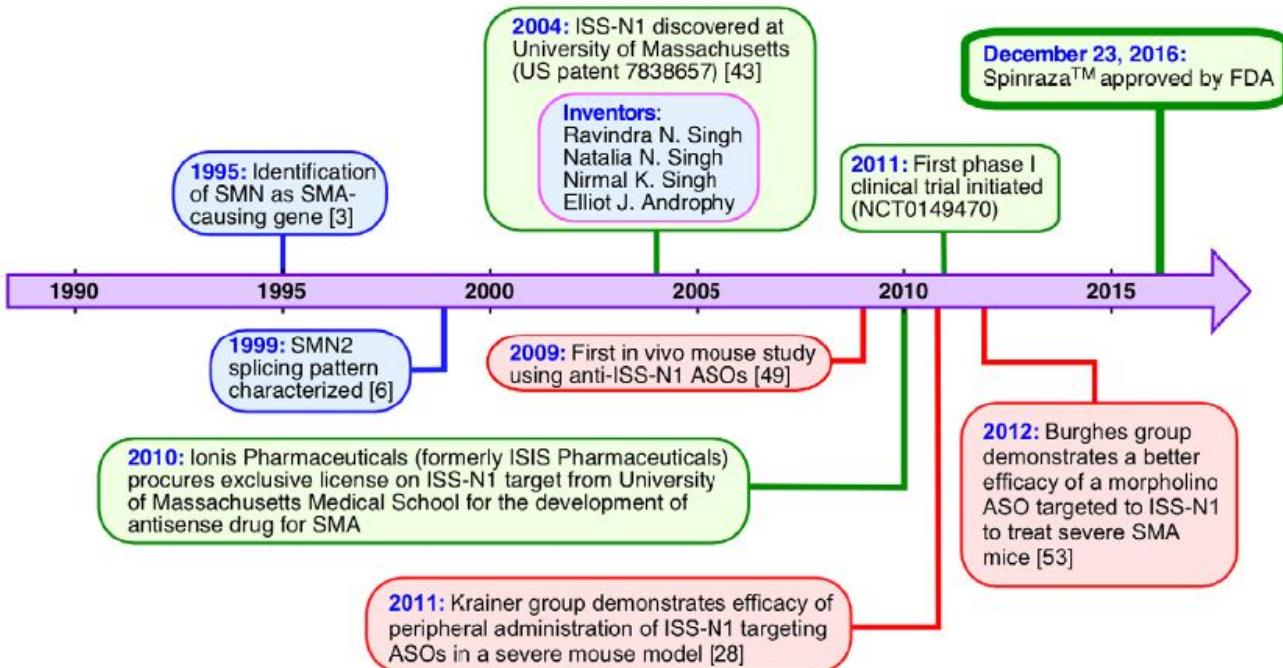
How Spinaraza (nusinersen) works, base by base

Nusinersen binds to ISS-N1, causing structural rearrangement and recruitment of U1 snRNP by TIA1.

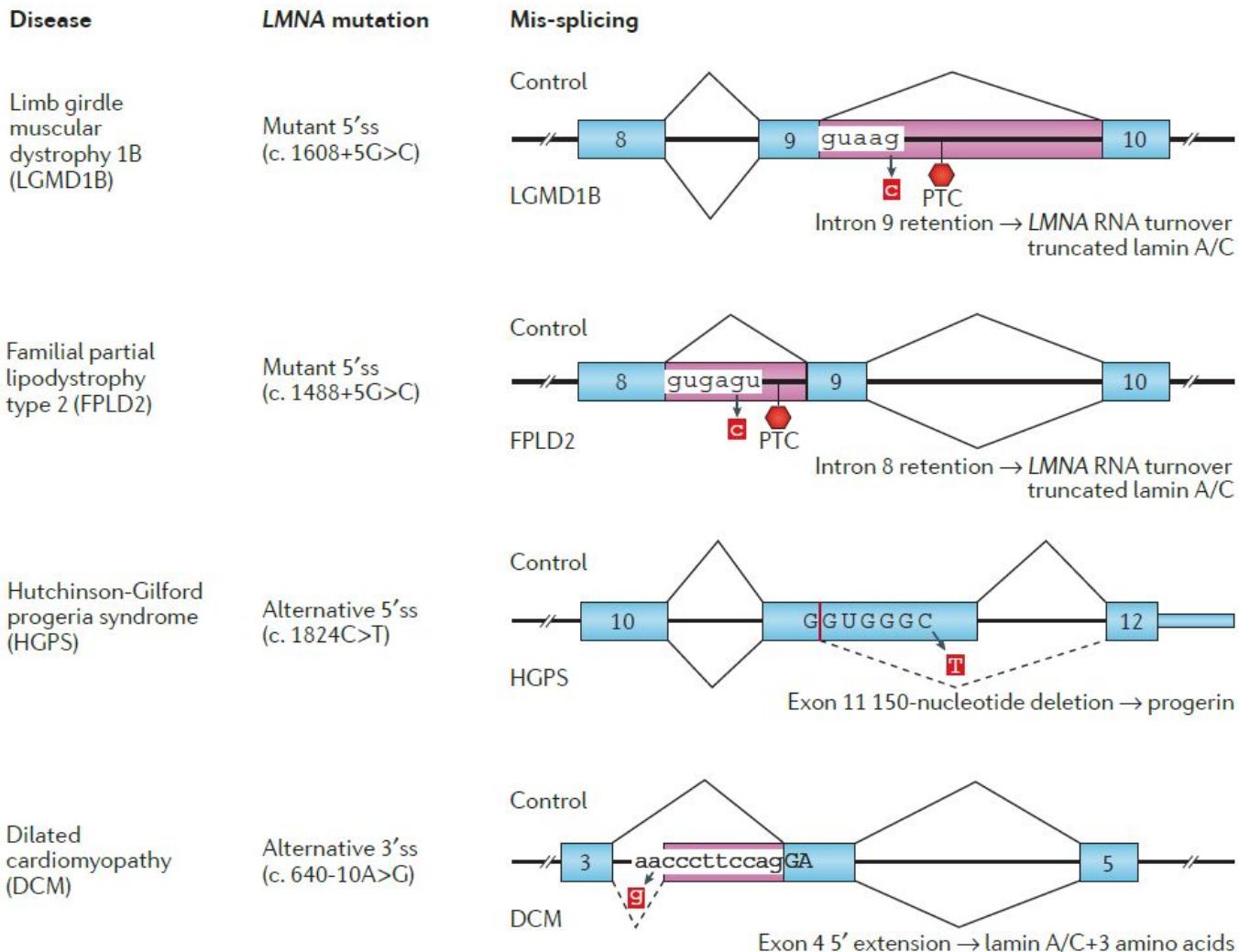
- ISS-N1: Intronic splicing silencer N1;
- TIA1: TIA1 cytotoxic granule associated RNA binding protein;
- TSLs: (inhibitory) terminal stem-loop structures;
- ISTL1: internal stem formed by a long-distance interaction



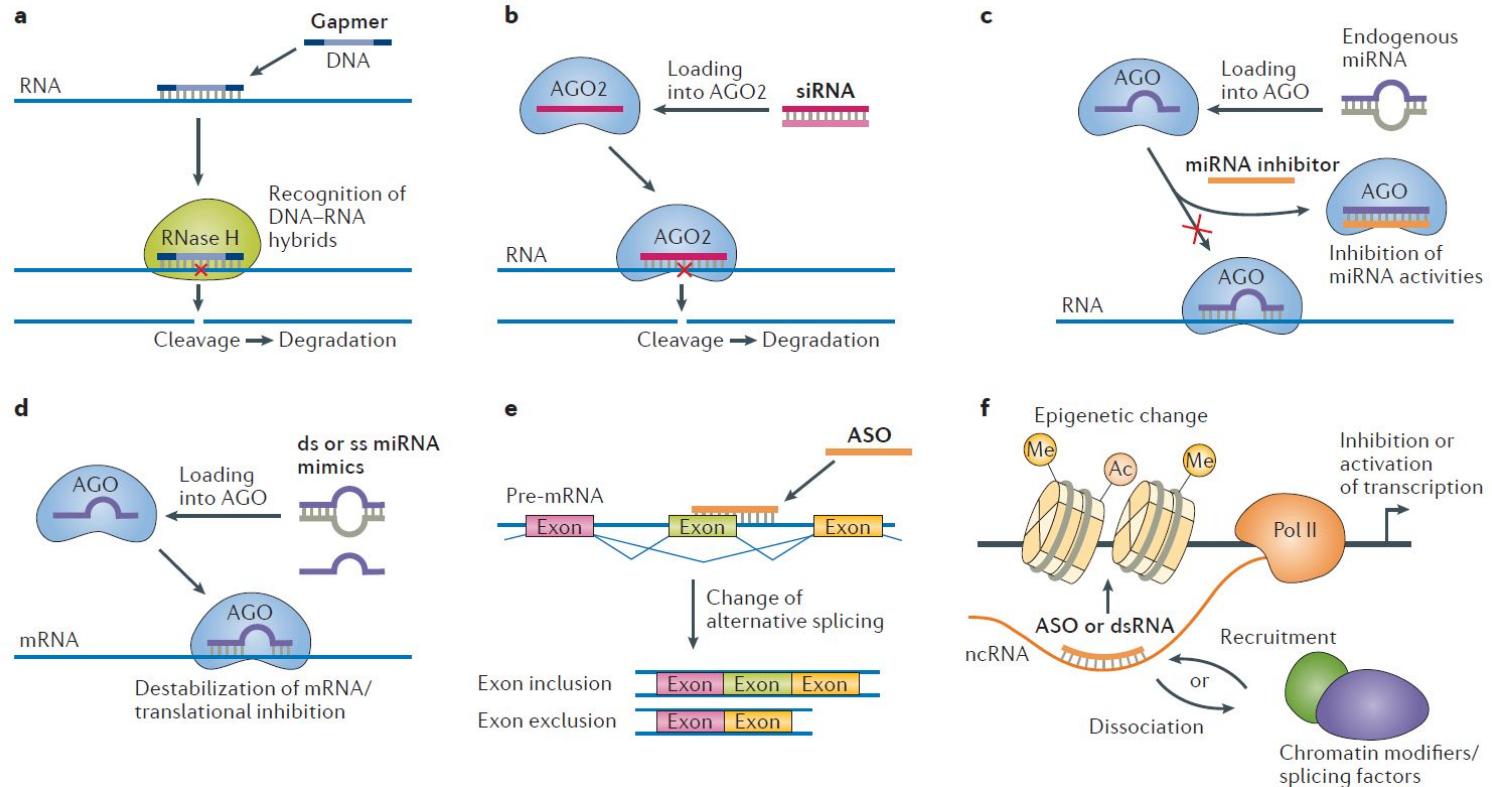
It takes 21 years to go from a molecular model to a population model



Splicing modifying oligo-nucleotide and other RNA therapeutics have strong disease relevances

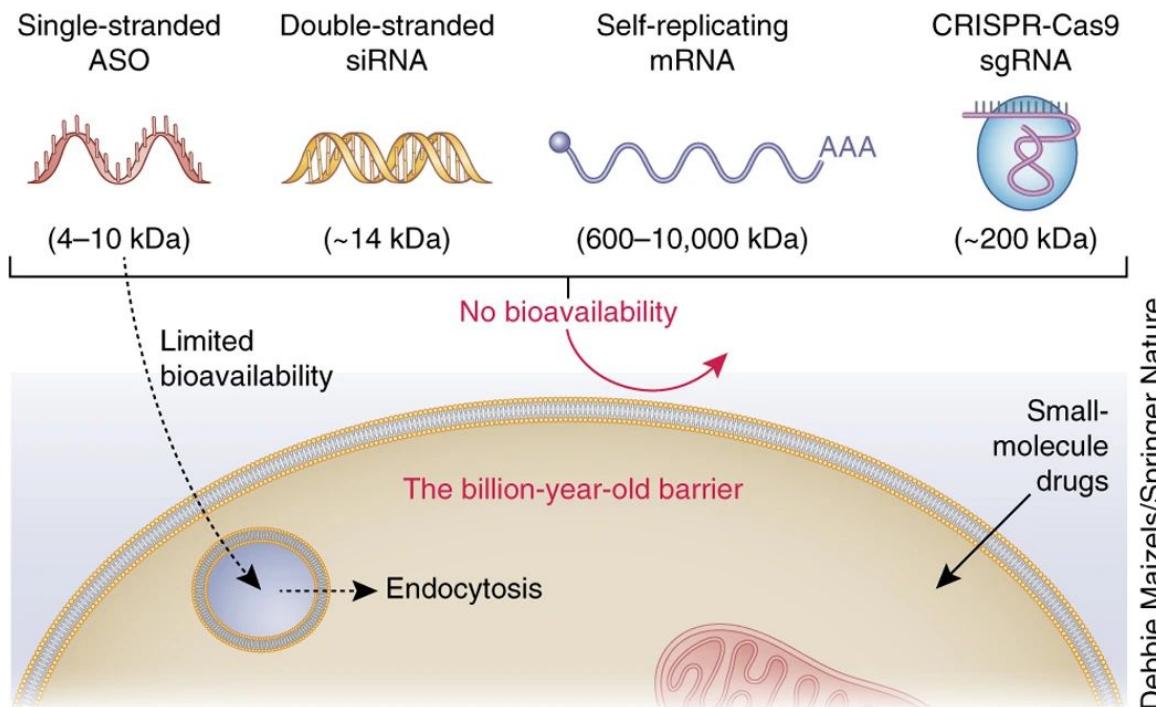


Regulating RNA levels or splicing with ASOs and duplex RNAs



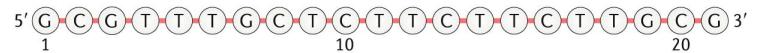
The four-billion-year-old barrier to RNA therapeutic

- Too large and charged to pass lipid bilayers
- Degradable by RNases
- Rapid clearance from liver and kidney
- Immunogenicity
- Endocytosis
- Delivery into organs other than liver and eye

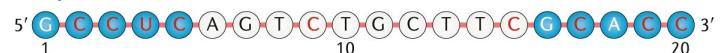


Chemistry of oligonucleotides evolves with time

a Fomivirsen



b Mipomersen



c Inotersen



d Eteplirsen



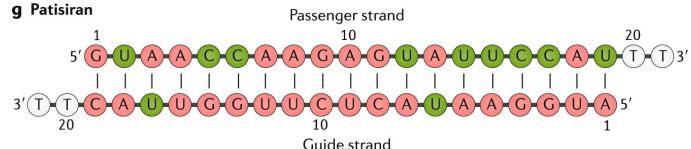
e Golodirsen



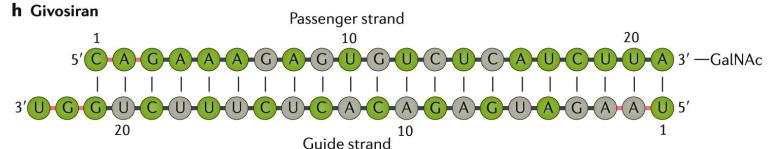
f Nusinersen



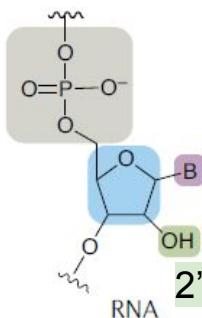
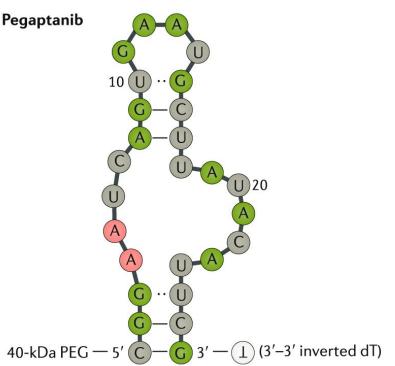
g Patisiran



h Givosiran



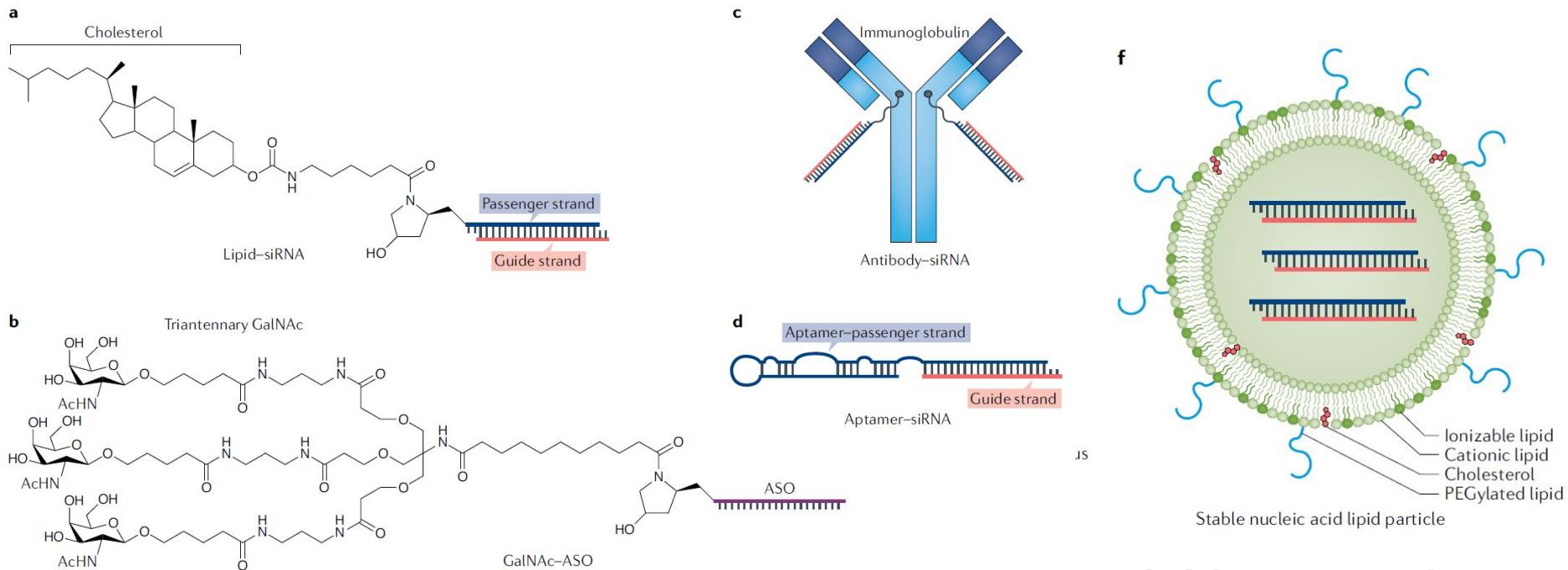
i Pegaptanib



- (N) DNA
- (N) RNA
- (N) PMO
- N 2'-O-methoxyethyl
- N 2'-O-methyl
- N 2'-Fluoro
- Y 5-Methyl pyrimidine
- Phosphorothioate
- Phosphodiester

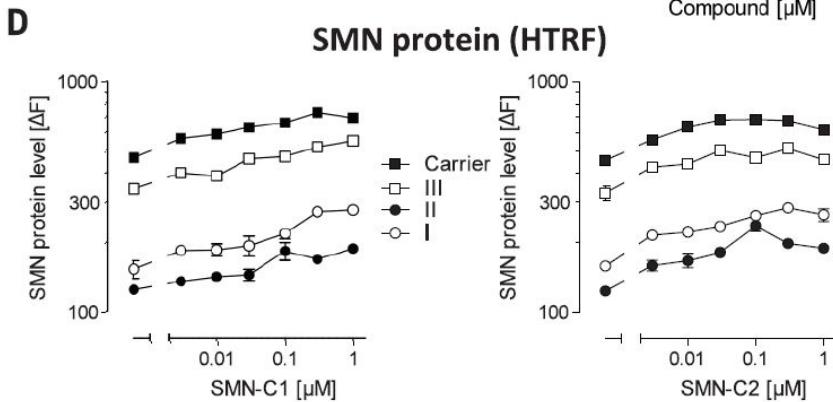
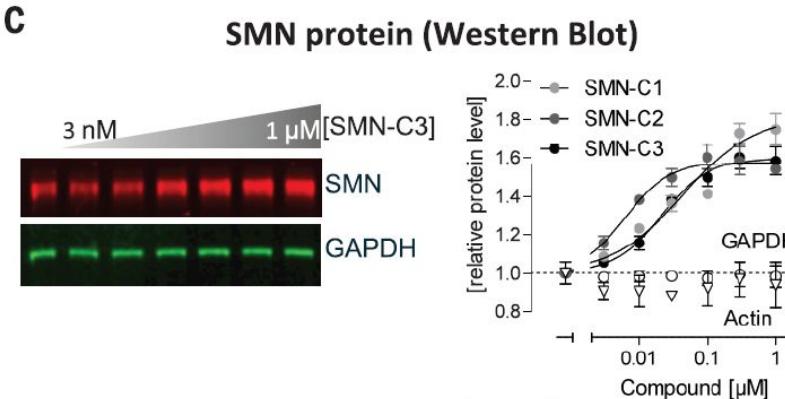
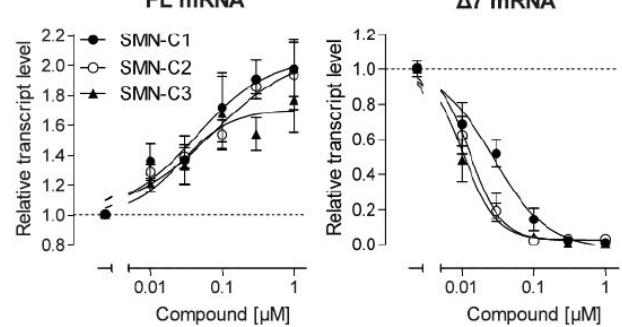
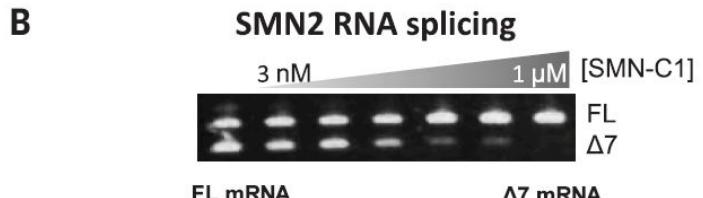
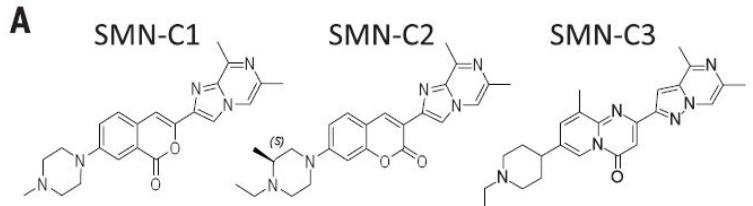
PMO=phosphorodiamidate morpholino oligomer

Delivery systems of antisense oligonucleotides



lipid nanoparticles

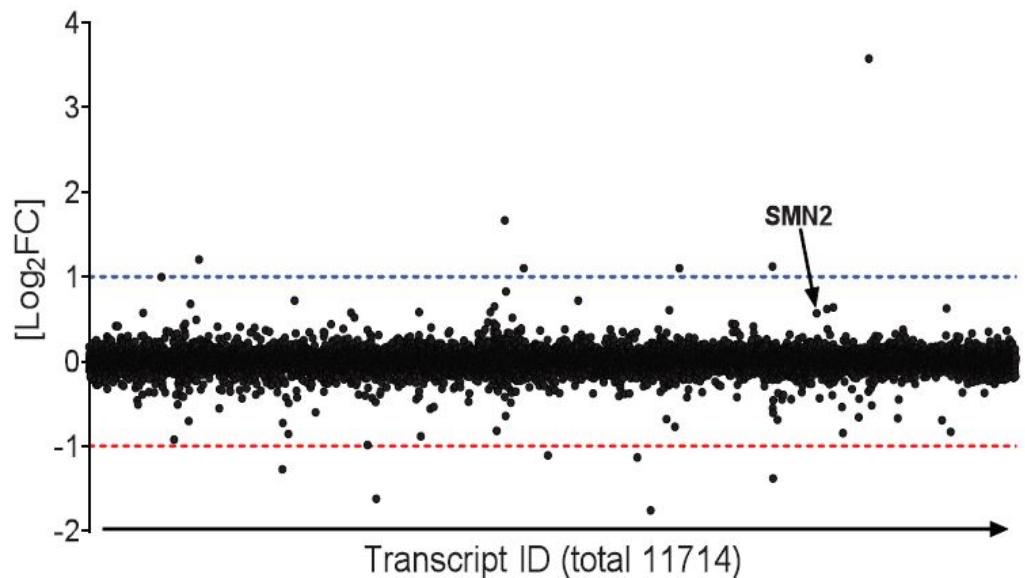
Small molecules as RNA splicing modifiers



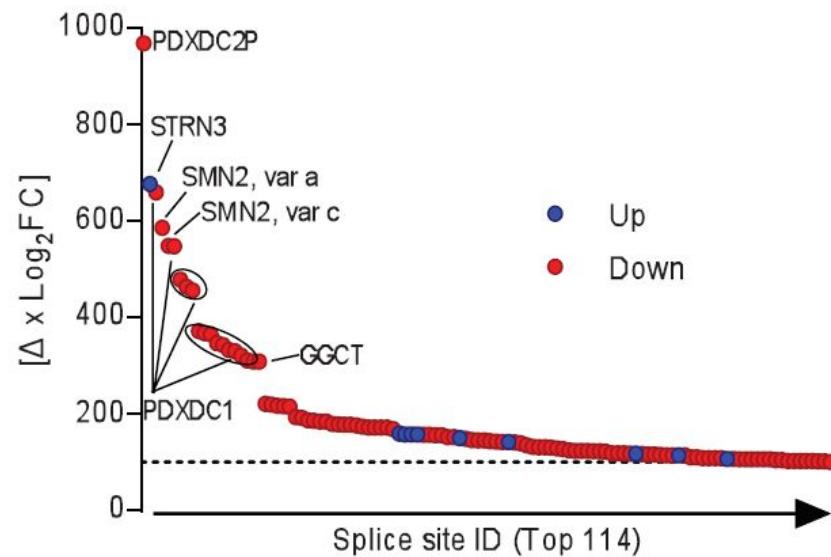
RNA sequencing confirms the specificity of SMN-C3

A

Transcriptional changes by SMN-C3

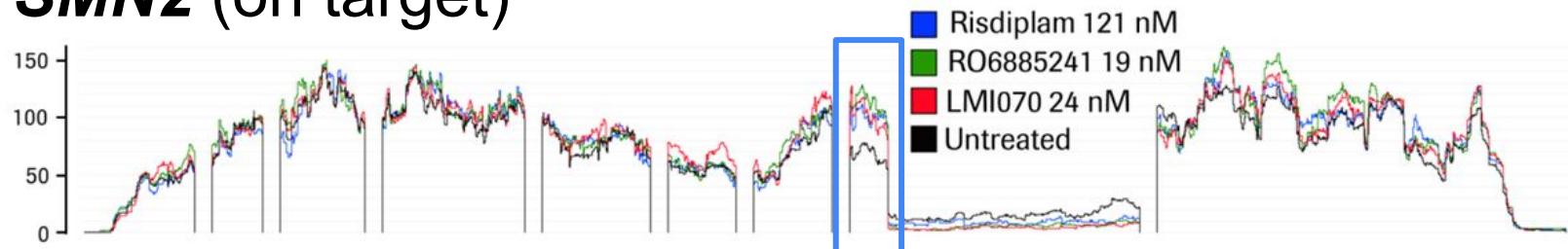

B

Splicing regulation by SMN-C3



RNA sequencing confirms the specificity of SMN-C3

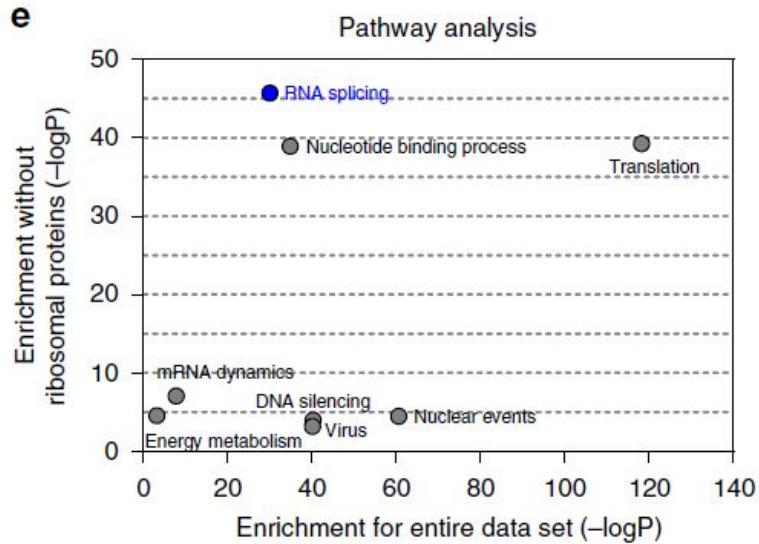
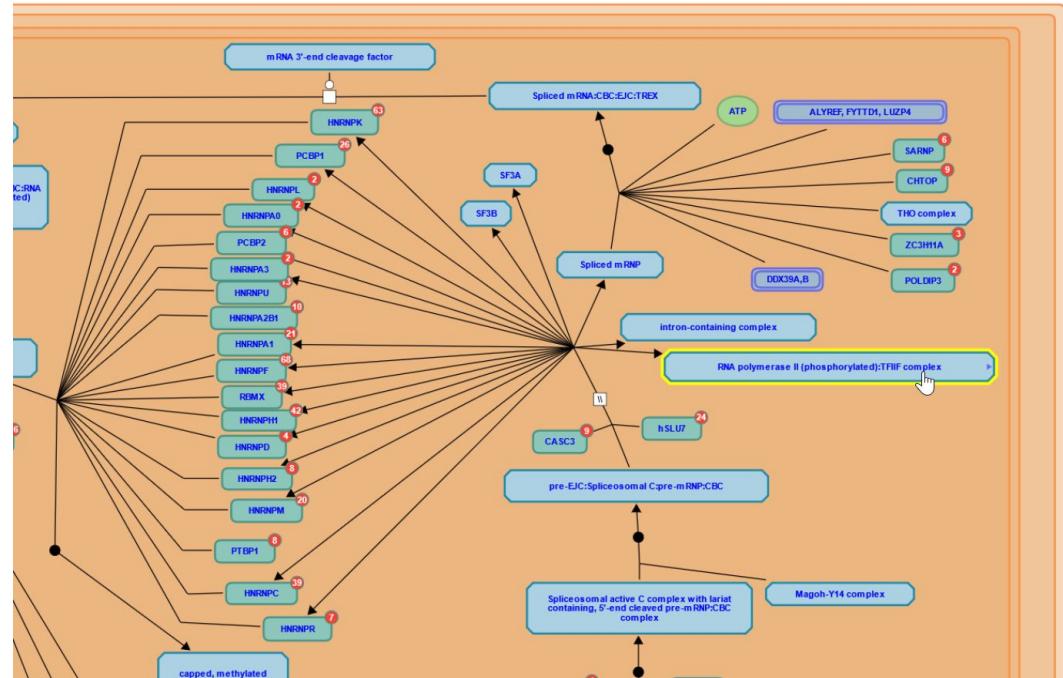
SMN2 (on target)



FOXM2 (off target)

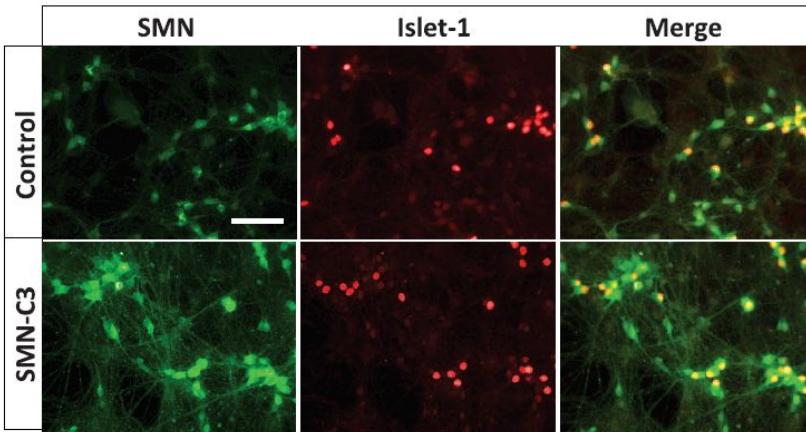


Gene-enrichment analysis confirms specific regulation of RNA splicing



Part of the mRNA splicing pathway in Reacome

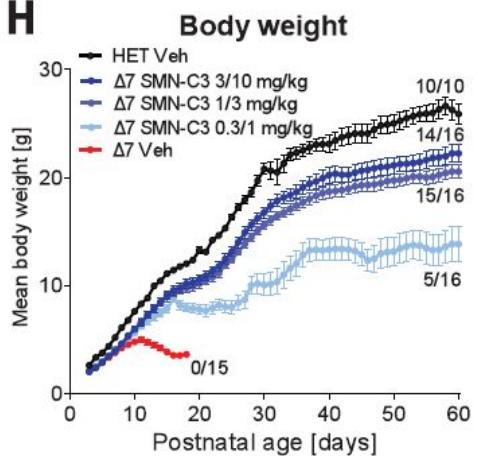
Experiments *in vitro* and *in vivo* support efficacy profiles of SMN-C3



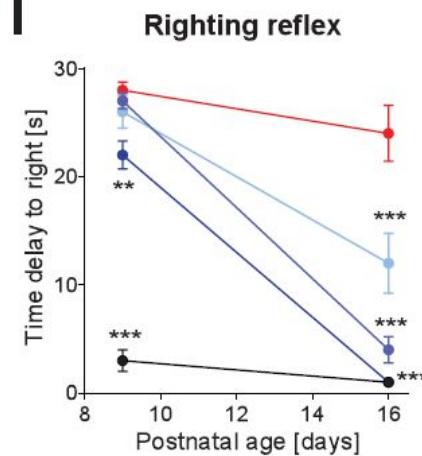
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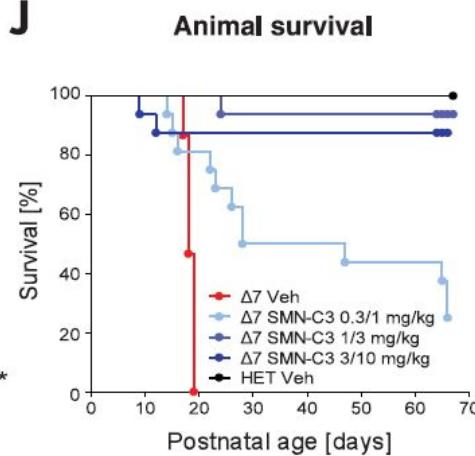
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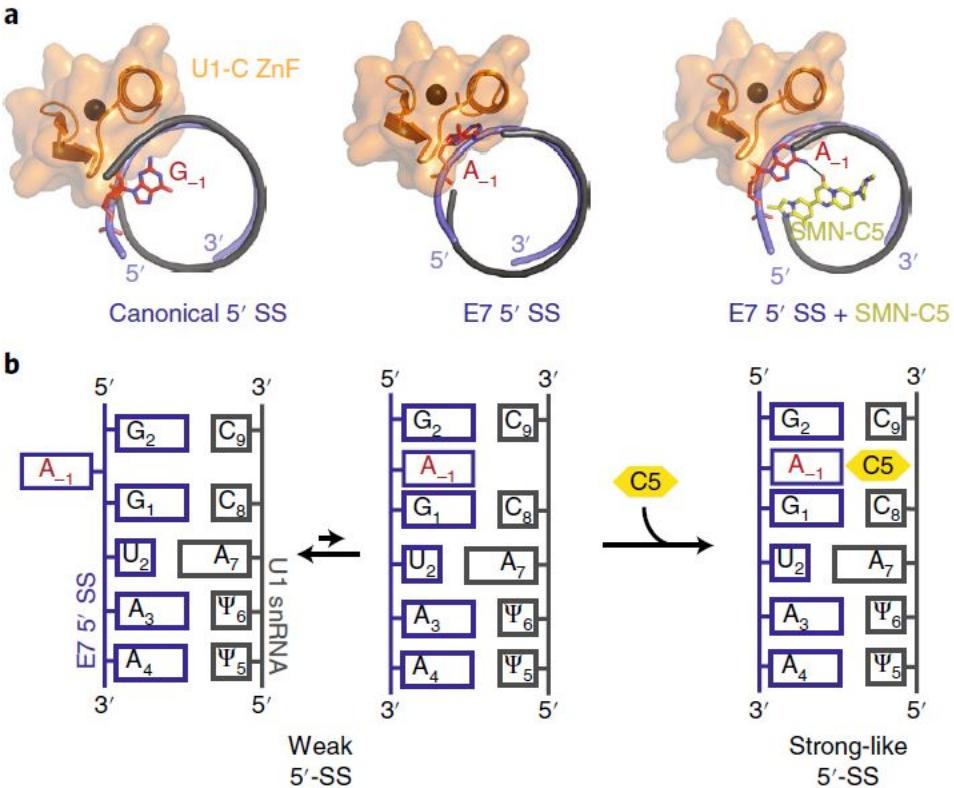
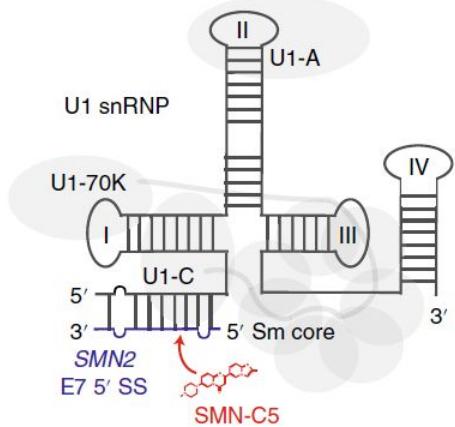
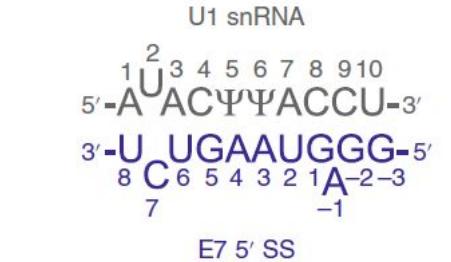
I

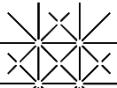


J



Structural basis of specific splicing correction





Clinical trial (FIREFISH Part 1) Results

Table 1. Demographic and Clinical Characteristics of the Patients at Baseline.*

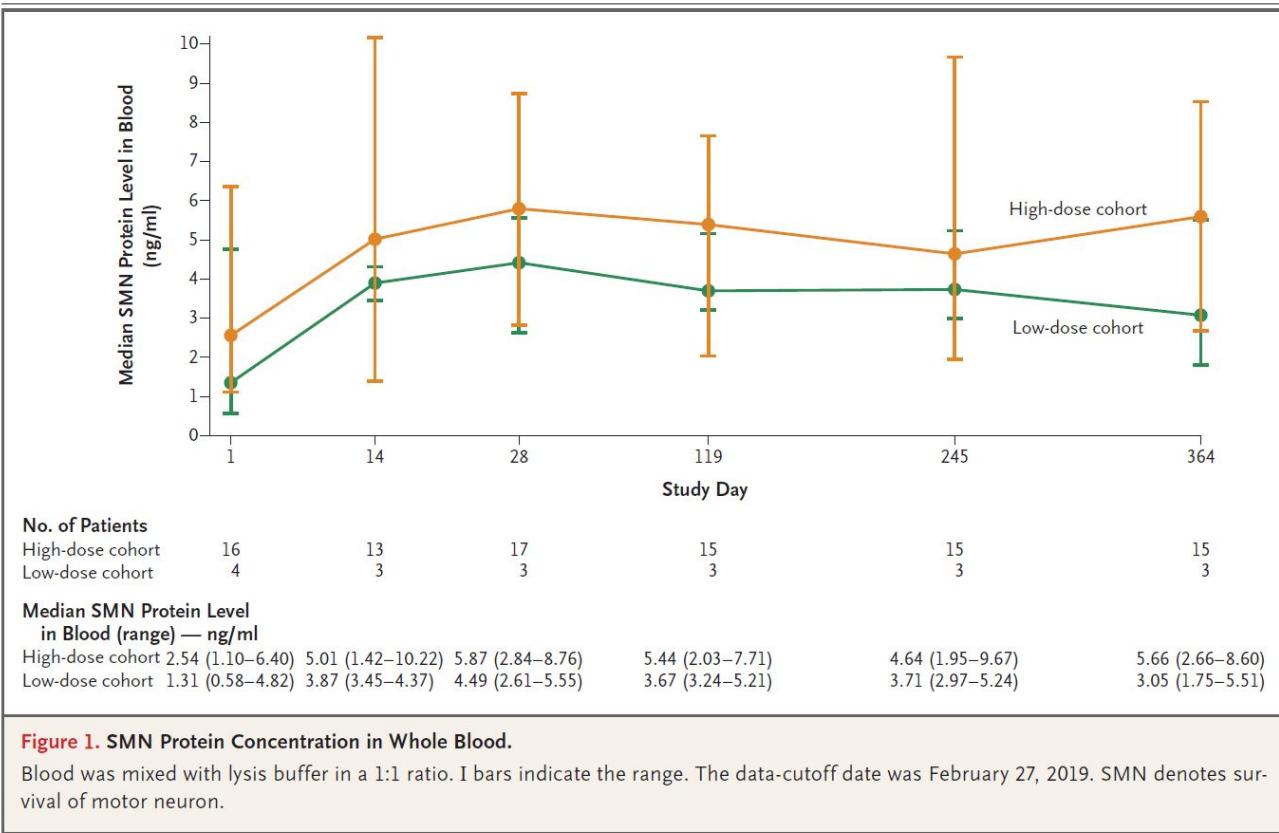
Characteristic	Low-Dose Cohort (N=4)	High-Dose Cohort (N=17)	All Infants (N=21)
Sex — no. (%)			
Female	4 (100)	11 (65)	15 (71)
Male	0	6 (35)	6 (29)
Median age (range) — mo			
At onset of symptoms	2.7 (2.0–3.0)	1.5 (0.9–3.0)	2.0 (0.9–3.0)
At diagnosis	3.3 (2.5–5.1)	3.0 (0.9–5.4)	3.0 (0.9–5.4)
At enrollment	6.9 (6.7–6.9)	6.3 (3.3–6.9)	6.7 (3.3–6.9)
Motor measures†			
Median CHOP-INTEND score (range)	23.5 (10–25)	24 (16–34)	24 (10–34)
Median HINE-2 score (range)	1 (0–3)	1 (0–2)	1 (0–3)
Respiratory support — no. (%)	0	5 (29)‡	5 (24)‡

Note: Table 2 is not complete

Table 2. Adverse Events.*

Event	Infants (N=21)
Total no. of adverse events	202
≥1 Adverse event — no. (%)	21 (100)
Total no. of serious adverse events	24
≥1 Serious adverse event — no. (%)	10 (48)
≥1 Adverse event of grade 3–5 — no. (%)	9 (43)
Serious adverse event with fatal outcome — no. (%)†	3 (14)
Most common adverse events — no. (%)‡	
Pyrexia	11 (52)
Upper respiratory tract infection	9 (43)
Diarrhea	6 (29)
Cough	5 (24)

Clinical trial (FIREFISH Part 1) Results



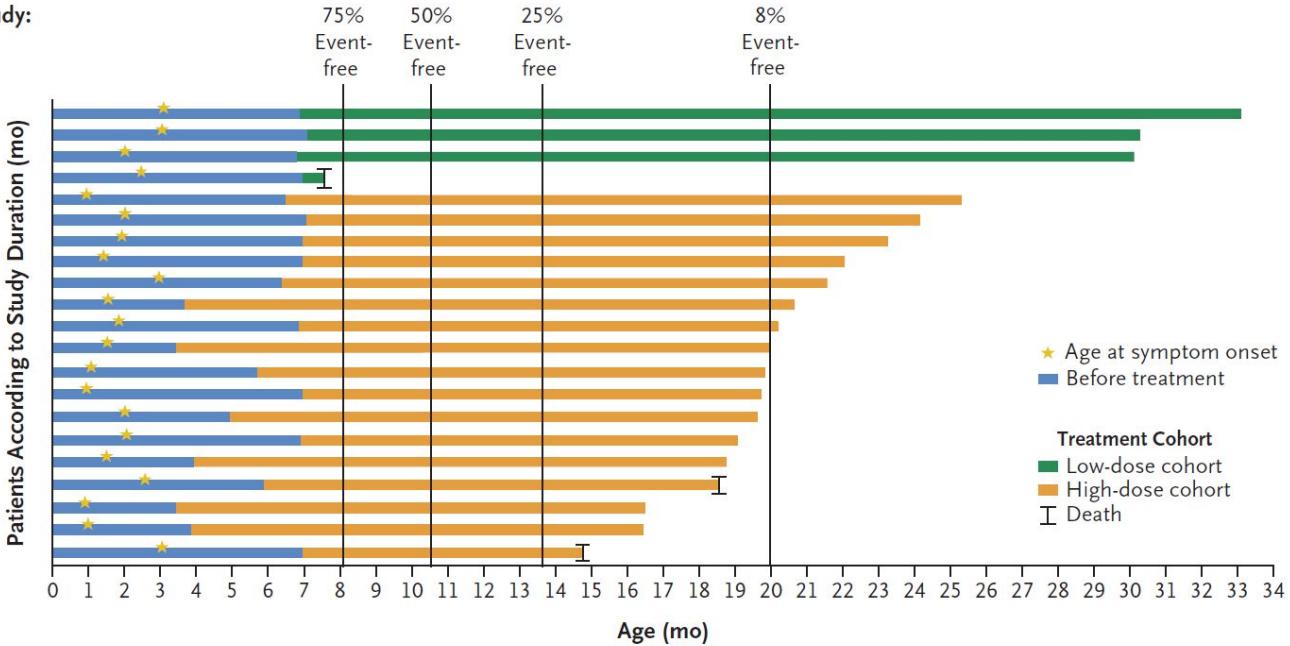
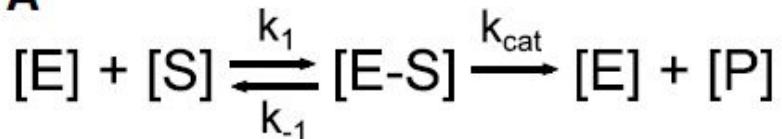
Natural History Study:


Figure 2. Event-free Survival.

Event-free survival was defined as being alive and not receiving permanent ventilation (tracheostomy or ventilation [bilevel positive airway pressure] for ≥ 16 hours per day continuously for > 3 weeks or continuous intubation for > 3 weeks, in the absence of, or after the resolution of, an acute reversible event). The percentages of patients who were event-free in a previous natural history study of spinal muscular atrophy⁷ are shown at the top of the graph for comparison. The median age at the combined outcome among patients in the previous study who had two copies of *SMN2* was 10.5 months (interquartile range, 8.1 to 13.6); event-free survival in that study was defined as being alive and not receiving noninvasive ventilation for 16 hours or more per day continuously for 2 or more weeks. The duration of our study was measured from the date of enrollment to the data-cutoff date. As of the data-cutoff date, three infants (one in the low-dose cohort and two in the high-dose cohort) had died; one additional infant in the high-dose cohort died after that date (Table S5).

Enzymic and genetic inhibition have distinct impact on reaction dynamics

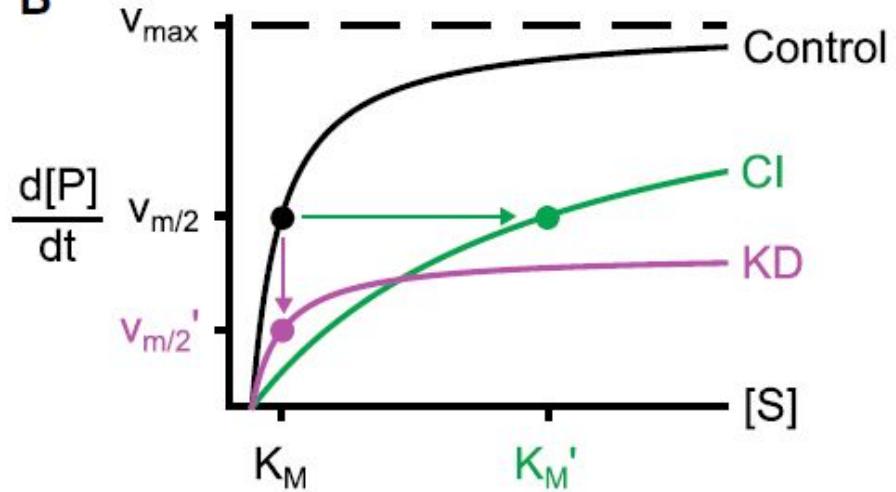
A



$$\frac{d[P]}{dt} = v_{\max} \frac{[S]}{[S] + K_M}$$

$$K_M = \frac{k_{-1} + k_{\text{cat}}}{k_1} \quad v_{\max} = k_{\text{cat}}[E]_o$$

B

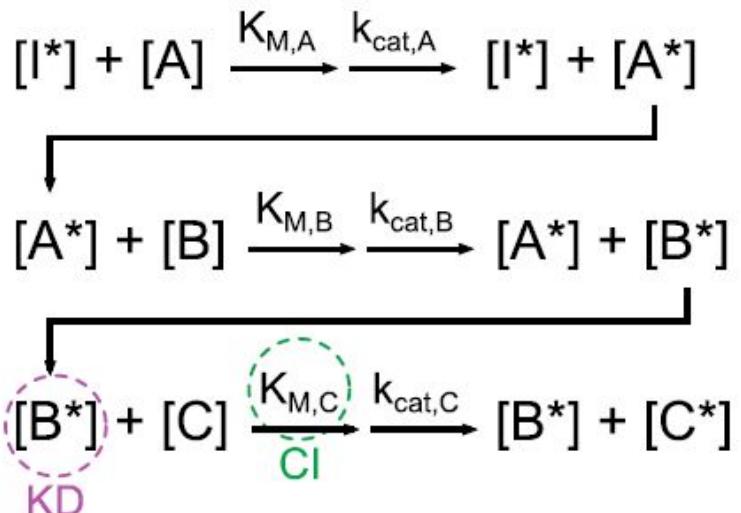


The Michaelis-Menten Equation

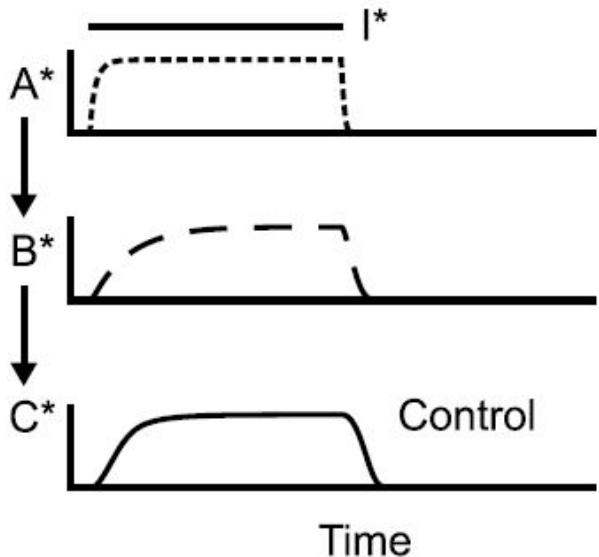
Competitive inhibition (CI)
versus knockdown (KD)

A linear system simulating enzymatic reactions

C



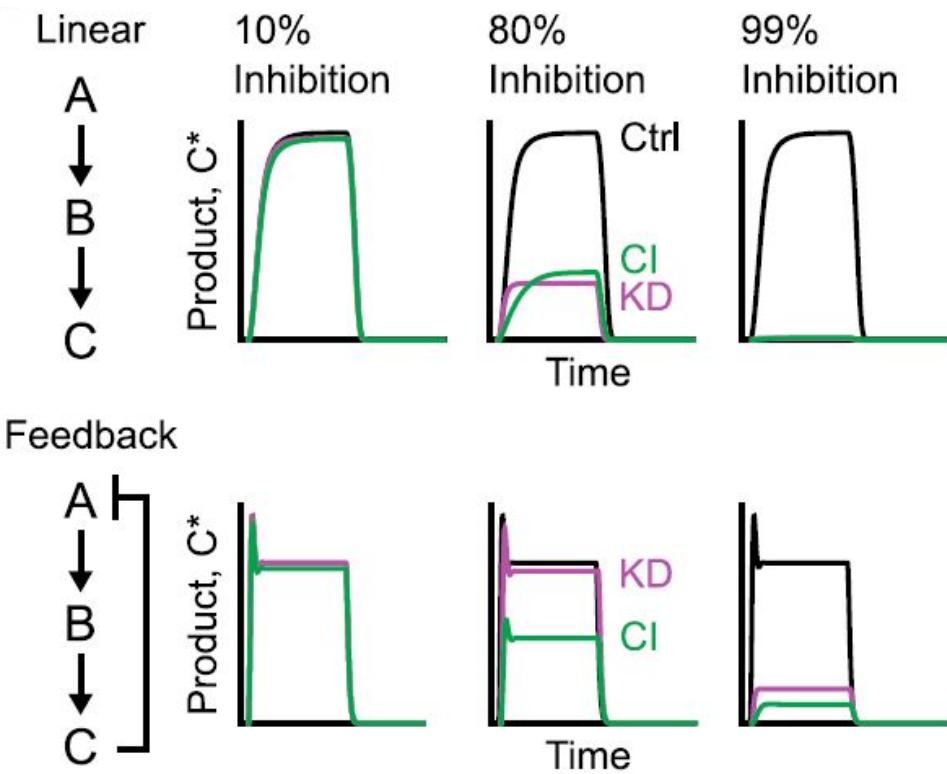
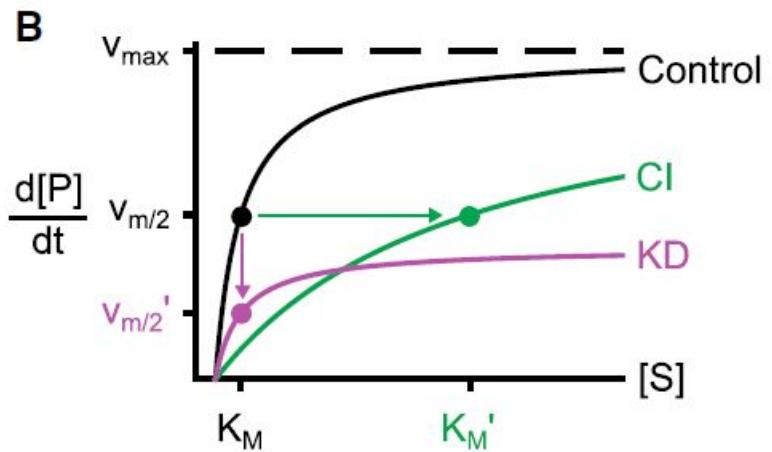
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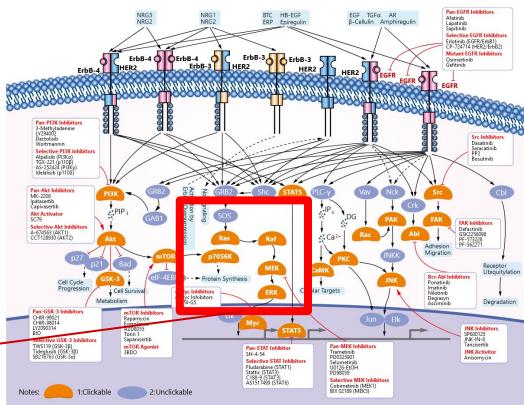
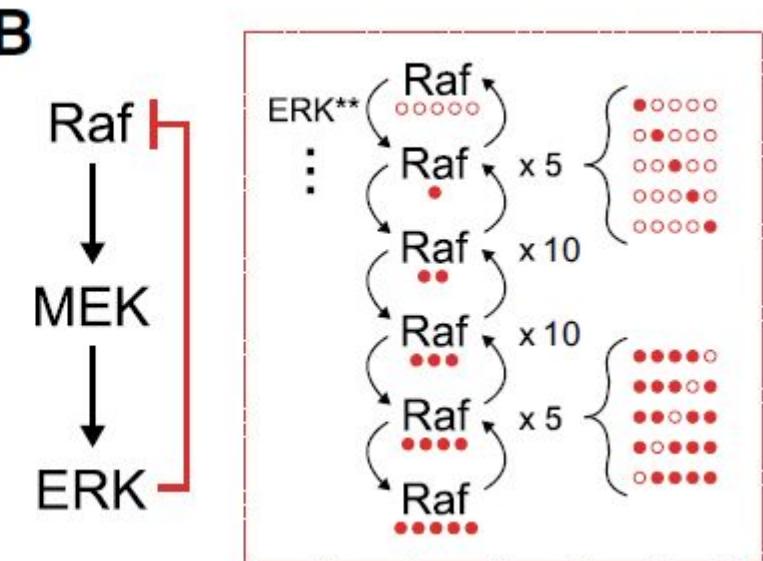
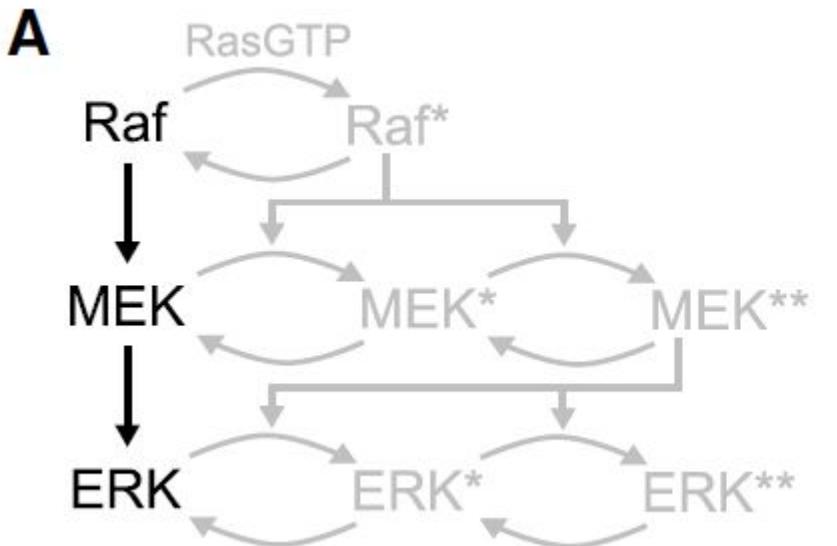
I^* : upstream input; A/A^* and B/B^* : inactivated and activated enzyme; C^* : product

Adding a negative feedback may differentiate effects of enzymatic and genetic inhibition

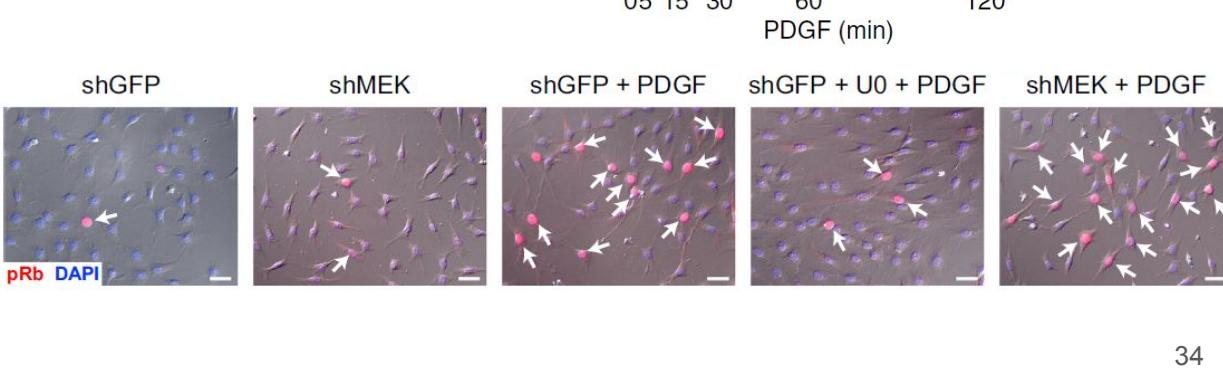
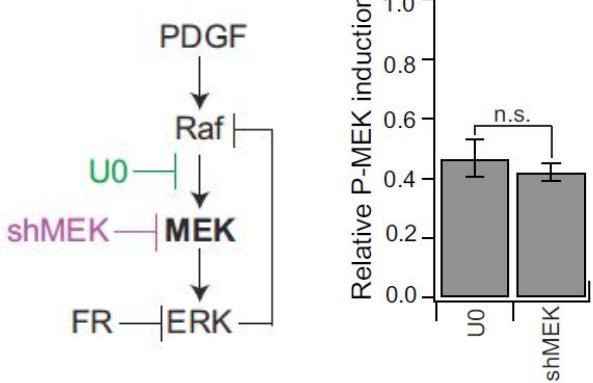
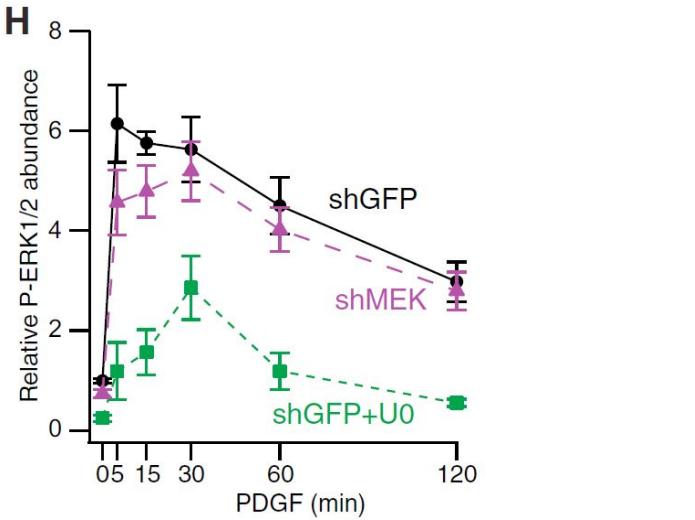
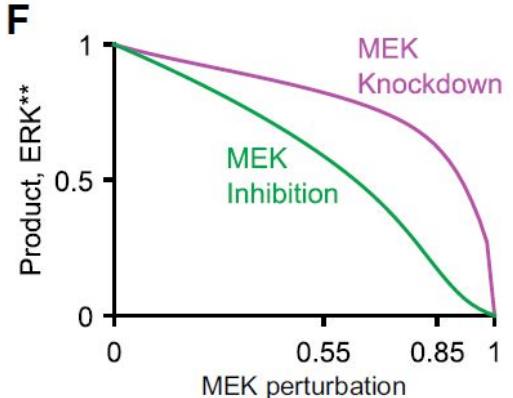
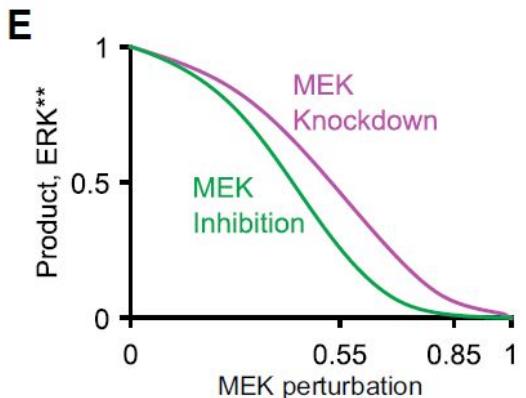
Intuition: when $[B^*]$ stays low, CI leads to **slower** accumulation of C^* than KD.



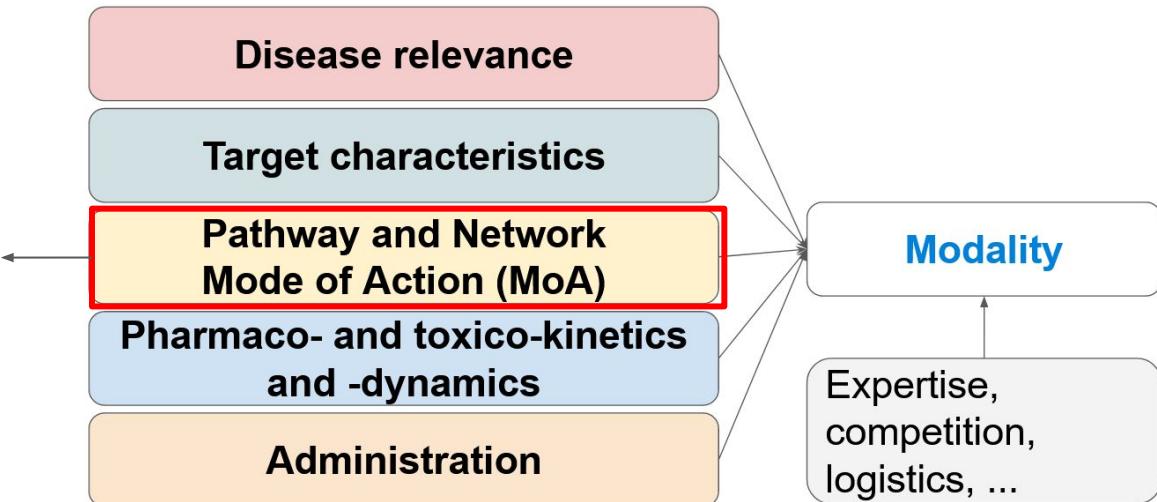
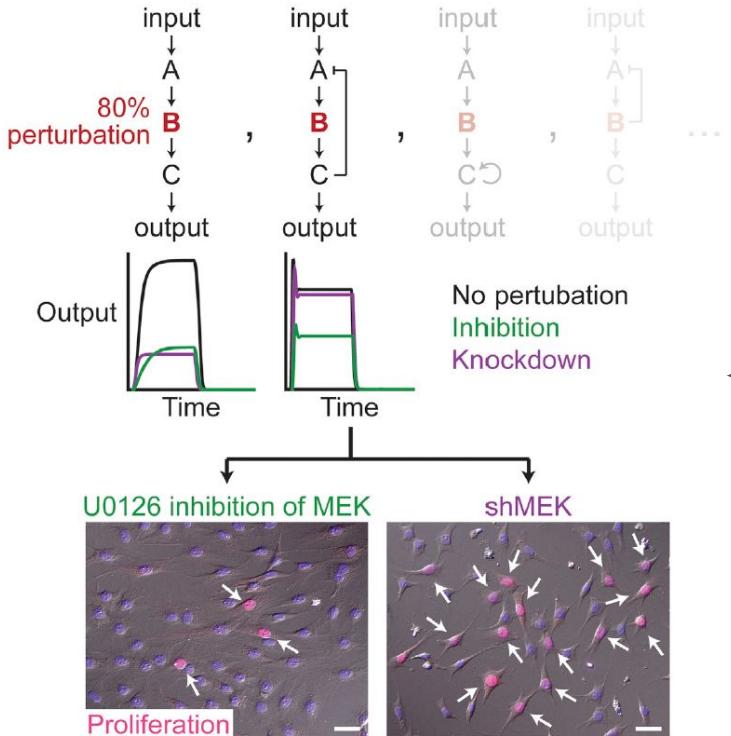
The MAPK/ERK pathway downstream of EGFR signalling



Confirmation of predicted difference of KD and CI



Computational biology may empower our choice of modality



Conclusions

- Given mechanistic understanding of biological processes underlying diseases, we can develop different modalities as therapeutics;
- Mathematical and computational biology
 - (1) helps with molecule design;
 - (2) reveals how drug candidate work and ranks them;
 - (3) contributes to modality selection;

Offline Activities

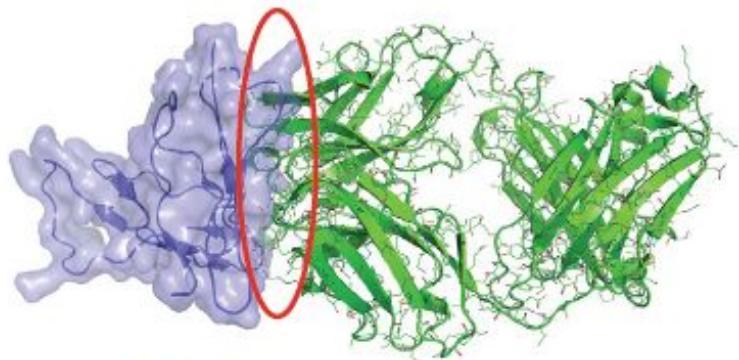
Reading about RNA therapies and vaccinations and build your own opinions about them:

- Levin, Arthur A. 2019. “Treating Disease at the RNA Level with Oligonucleotides.” New England Journal of Medicine 380 (1): 57–70. <https://doi.org/10.1056/NEJMra1705346>.
- Oligonucleotides and their discontents by Derek Loewe in *In The Pipeline*:
<https://blogs.sciencemag.org/pipeline/archives/2021/03/24/oligonucleotides-and-their-discontents>
- The next act for messenger RNA could be bigger than covid vaccines, MIT Technology Review 2021, by Antonio Regalado,
<https://www.technologyreview.com/2021/02/05/1017366/messenger-rna-vaccines-covid-hiv/>

Multispecific Drug Use or Target Interactions

b Conventional drug:

- Forms 1 drug–target interface
- Can act throughout body
- Only works if its binding to target alters function of target



IL-2R α

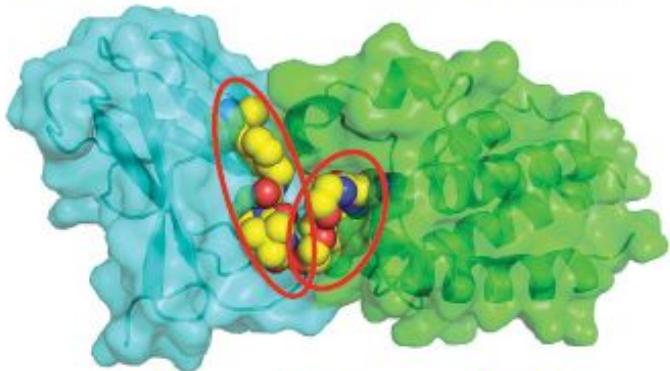
Basiliximab

c Obligate multispecific drug:

- Forms 2 or more drug–target interfaces

Class 1 'tetherbodies'
 • Enrich drug at relevant site of action

Class 2 'matchmakers'
 • Link drug to a biological effector



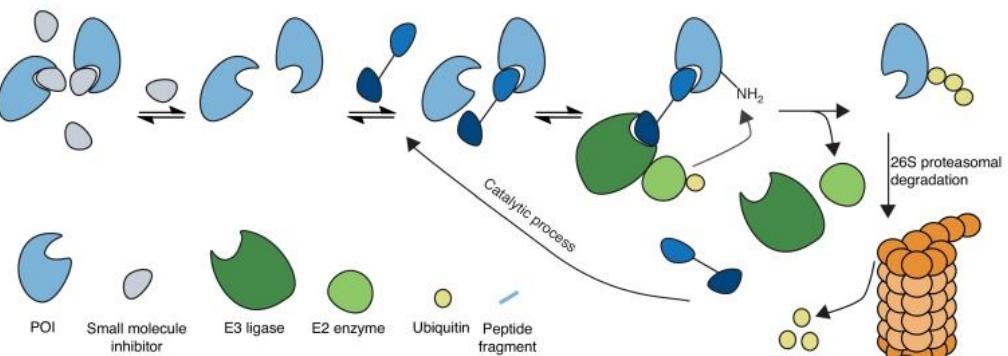
VHL

MZ1

BRD4^{BD2}

(a) Occupancy-driven pharmacology

Protein function is modulated via inhibition

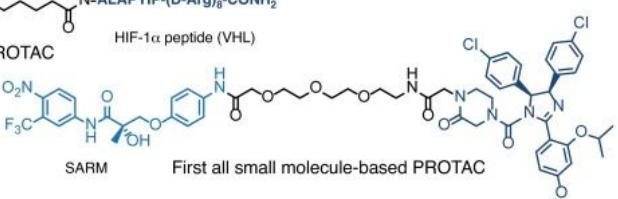
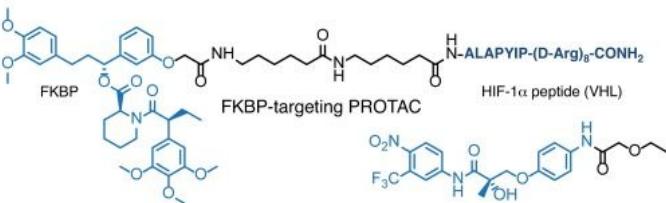


(b) Event-driven pharmacology

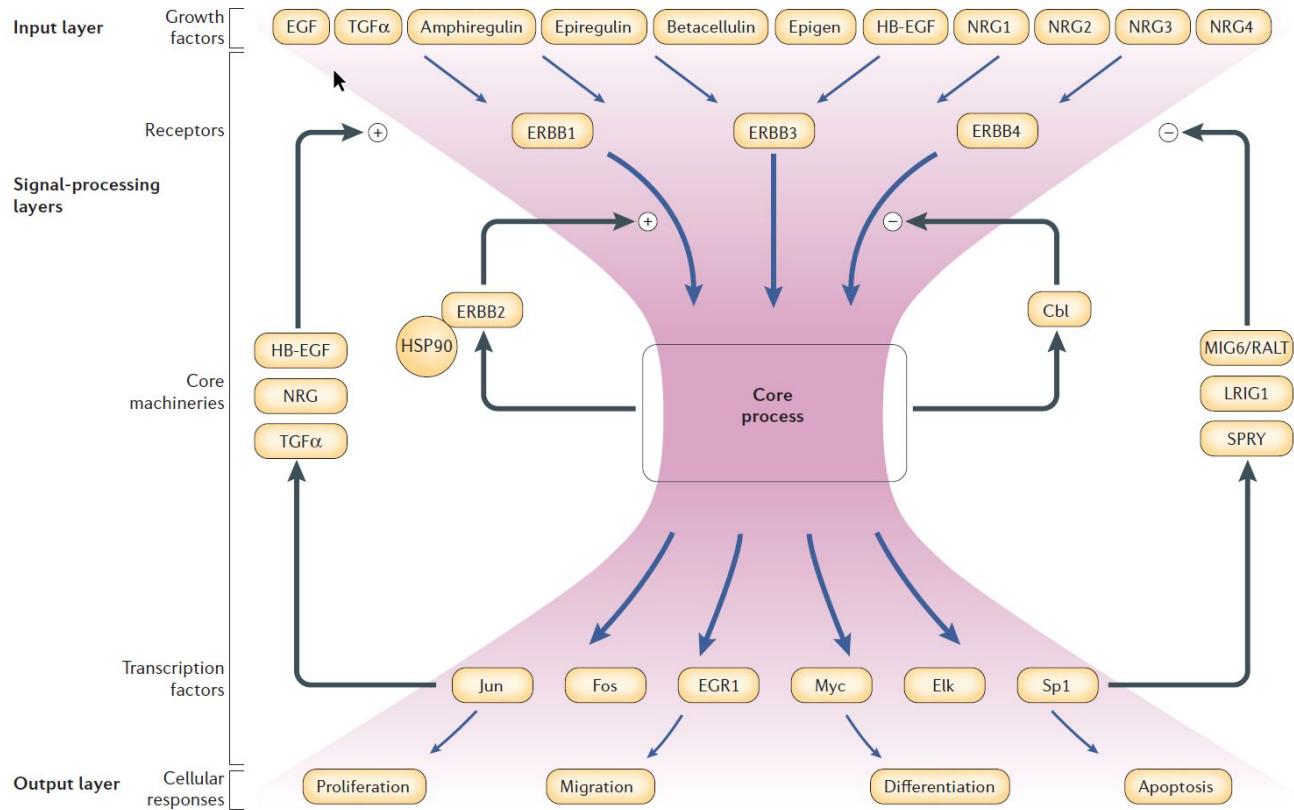
Protein function is modulated via PROTAC induced degradation



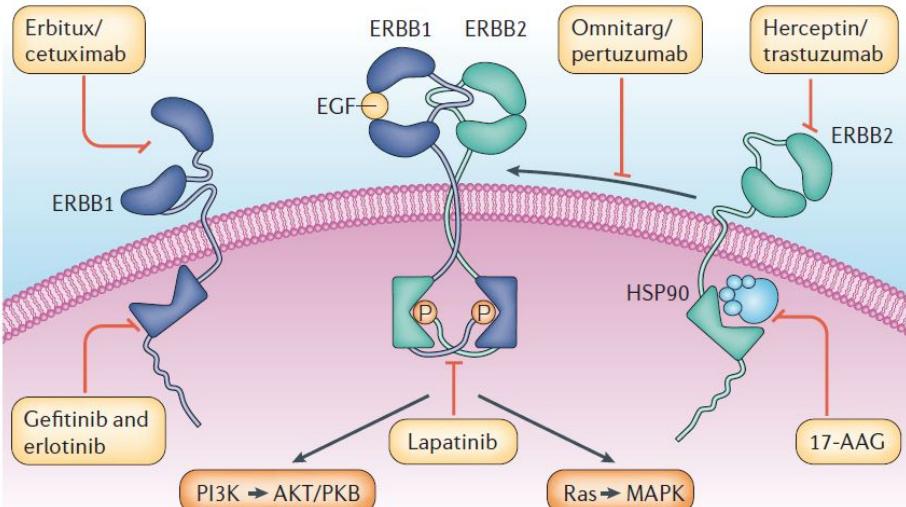
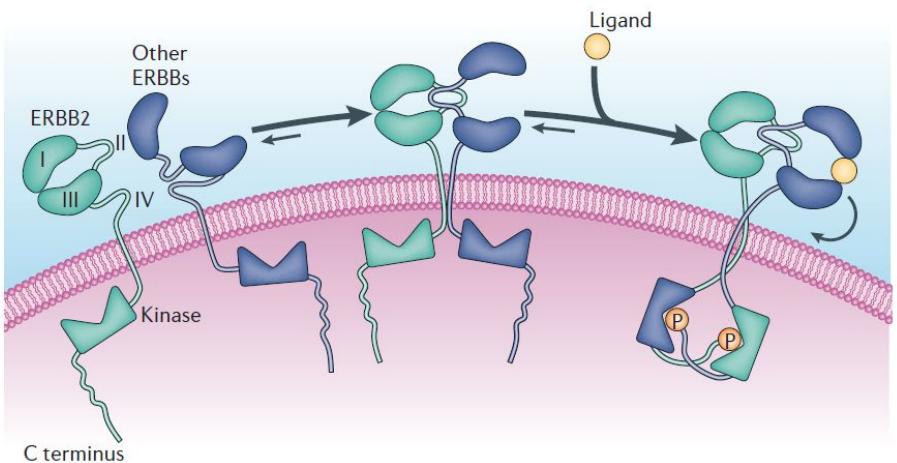
(c) PROteolysis TArgeting Chimera (PROTAC)



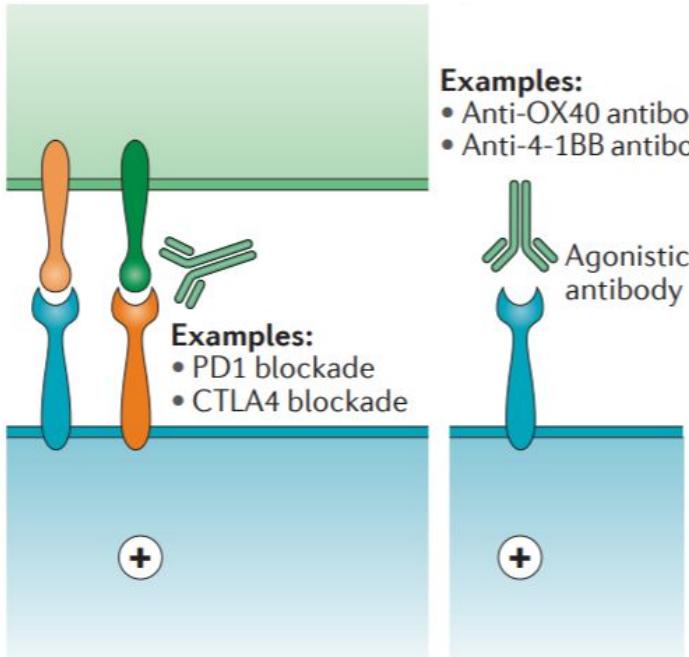
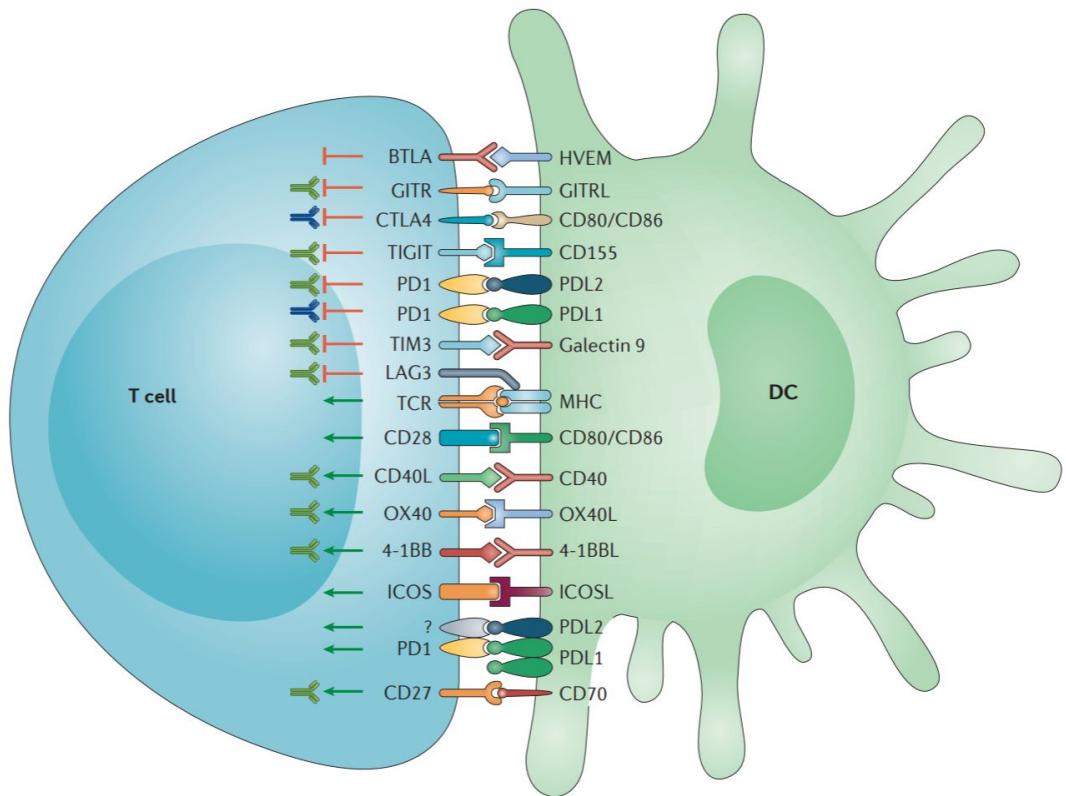
The Bow-Tie model of signaling transduction



ERBB signaling system and antibody drugs



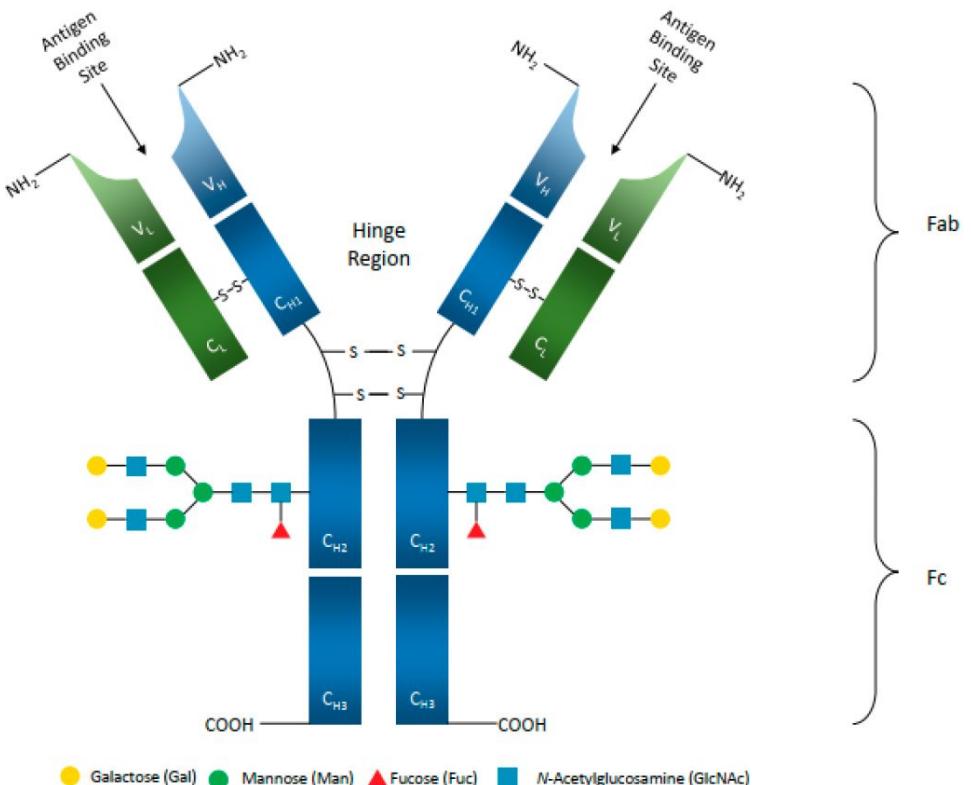
Immune checkpoints as drug targets



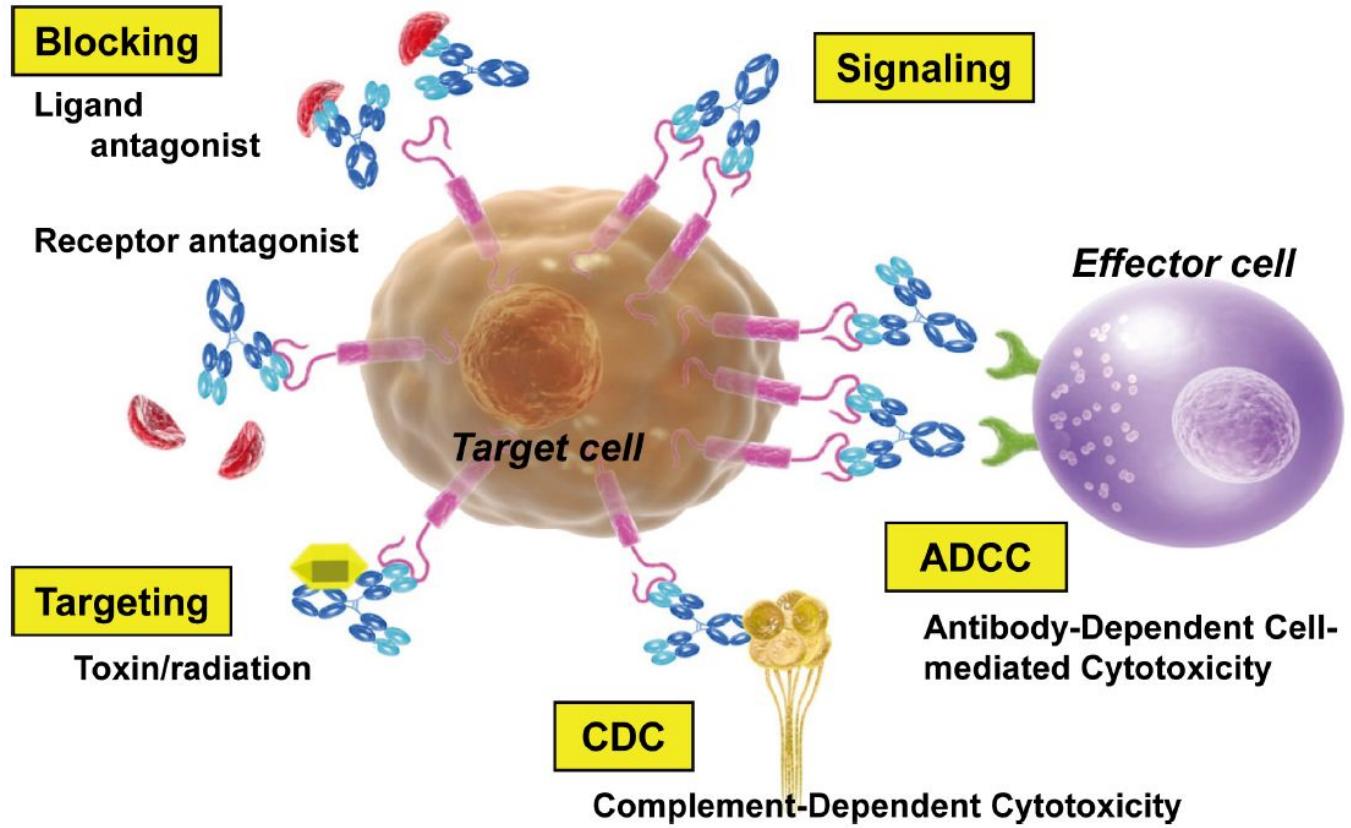
Peptide and antibody

- Antibody design
- Immunogenicity

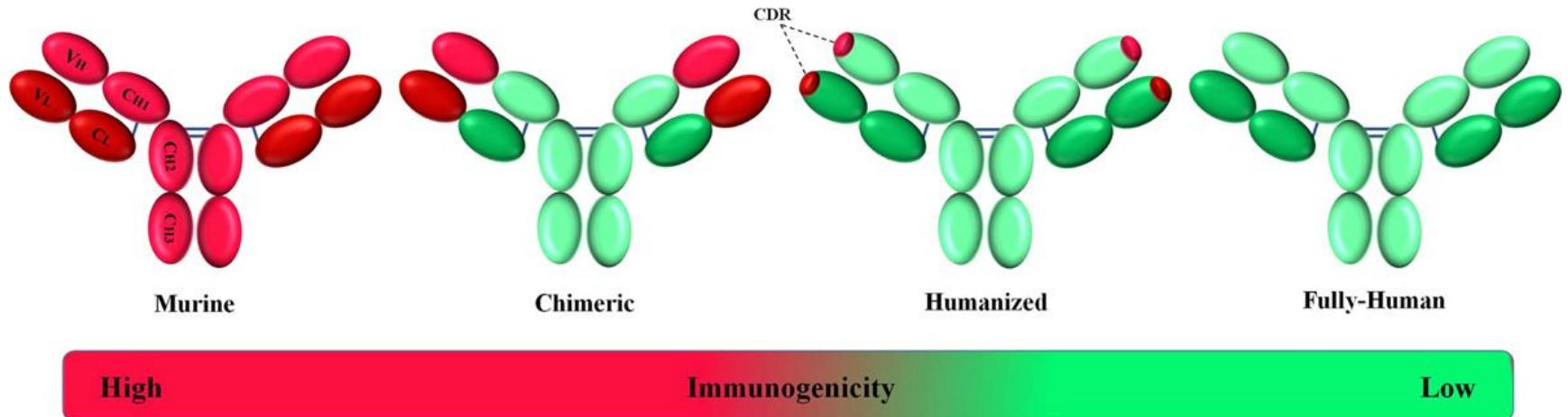
Structure of antibodies



Mechanisms of action of therapeutic antibodies



Evolution of therapeutic antibodies



References

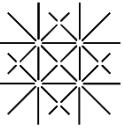
1. Valeur, Eric, Stéphanie M. Guéret, Hélène Adihou, Ranganath Gopalakrishnan, Malin Lemurell, Herbert Waldmann, Tom N. Grossmann, and Alleyn T. Plowright. 2017. "New Modalities for Challenging Targets in Drug Discovery." *Angewandte Chemie International Edition* 56 (35): 10294–323. <https://doi.org/10.1002/anie.201611914>.
2. Naryshkin, N. A., M. Weetall, A. Dakka, J. Narasimhan, X. Zhao, Z. Feng, K. K. Y. Ling, et al. 2014. "SMN2 Splicing Modifiers Improve Motor Function and Longevity in Mice with Spinal Muscular Atrophy." *Science* 345 (6197): 688–93. <https://doi.org/10.1126/science.1250127>.
3. Sivaramakrishnan, Manaswini, Kathleen D. McCarthy, Sébastien Campagne, Sylwia Huber, Sonja Meier, Angélique Augustin, Tobias Heckel, et al. 2017. "Binding to SMN2 Pre-MRNA-Protein Complex Elicits Specificity for Small Molecule Splicing Modifiers." *Nature Communications* 8 (November): 1476. <https://doi.org/10.1038/s41467-017-01559-4>.
4. Ratni, Hasane, Martin Ebeling, John Baird, Stefanie Bendels, Johan Bylund, Karen S. Chen, Nora Denk, et al. 2018. "Discovery of Risdiplam, a Selective Survival of Motor Neuron-2 (SMN2) Gene Splicing Modifier for the Treatment of Spinal Muscular Atrophy (SMA)." *Journal of Medicinal Chemistry* 61 (15): 6501–17. <https://doi.org/10.1021/acs.jmedchem.8b00741>.
5. Hagedorn, Peter H., Malene Pontoppidan, Tina S. Bisgaard, Marco Berrera, Andreas Dieckmann, Martin Ebeling, Marianne R. Møller, et al. 2018. "Identifying and Avoiding Off-Target Effects of RNase H-Dependent Antisense Oligonucleotides in Mice." *Nucleic Acids Research* 46 (11): 5366–80. <https://doi.org/10.1093/nar/gky397>.
6. Ding, Yu, Yitian Fei, and Boxun Lu. 2020. "Emerging New Concepts of Degrader Technologies." *Trends in Pharmacological Sciences* 41 (7): 464–74. <https://doi.org/10.1016/j.tips.2020.04.005>.
7. Donovan, Katherine A., Fleur M. Ferguson, Jonathan W. Bushman, Nicholas A. Eleuteri, Debabrata Bhunia, SeongShick Ryu, Li Tan, et al. 2020. "Mapping the Degradable Kinome Provides a Resource for Expedited Degrader Development." *Cell* 183 (6): 1714–1731.e10. <https://doi.org/10.1016/j.cell.2020.10.038>.
8. Ottis, Philipp, Chiara Palladino, Phillip Thienger, Adrian Britschgi, Christian Heichinger, Marco Berrera, Alice Julien-Laferriere, et al. 2019. "Cellular Resistance Mechanisms to Targeted Protein Degradation Converge Toward Impairment of the Engaged Ubiquitin Transfer Pathway." *ACS Chemical Biology* 14 (10): 2215–23. <https://doi.org/10.1021/acscchembio.9b00525>.

References (continued)

9. Stanton, Benjamin Z., Emma J. Chory, and Gerald R. Crabtree. 2018. "Chemically Induced Proximity in Biology and Medicine." *Science* 359 (6380): eaao5902. <https://doi.org/10.1126/science.aao5902>.
10. Baran, Dror, M. Gabriele Pszolla, Gideon D. Lapidoth, Christoffer Norn, Orly Dym, Tamar Unger, Shira Albeck, Michael D. Tyka, and Sarel J. Fleishman. 2017. "Principles for Computational Design of Binding Antibodies." *Proceedings of the National Academy of Sciences* 114 (41): 10900–905. <https://doi.org/10.1073/pnas.1707171114>.
11. Jain, Tushar, Tingwan Sun, Stéphanie Durand, Amy Hall, Nga Rewa Houston, Juergen H. Nett, Beth Sharkey, et al. 2017. "Biophysical Properties of the Clinical-Stage Antibody Landscape." *Proceedings of the National Academy of Sciences* 114 (5): 944–49. <https://doi.org/10.1073/pnas.1616408114>.
12. Saka, Koichiro, Taro Kakuzaki, Shoichi Metsugi, Daiki Kashiwagi, Kenji Yoshida, Manabu Wada, Hiroyuki Tsunoda, and Reiji Teramoto. 2021. "Antibody Design Using LSTM Based Deep Generative Model from Phage Display Library for Affinity Maturation." *Scientific Reports* 11 (1): 5852. <https://doi.org/10.1038/s41598-021-85274-7>.
13. Shirai, Hiroki, Catherine Prades, Randi Vita, Paolo Marcatili, Bojana Popovic, Jianqing Xu, John P. Overington, et al. 2014. "Antibody Informatics for Drug Discovery." *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics, Recent advances in molecular engineering of antibody*, 1844 (11): 2002–15. <https://doi.org/10.1016/j.bbapap.2014.07.006>.
14. Muttenthaler, Markus, Glenn F. King, David J. Adams, and Paul F. Alewood. 2021. "Trends in Peptide Drug Discovery." *Nature Reviews Drug Discovery* 20 (4): 309–25. <https://doi.org/10.1038/s41573-020-00135-8>.
15. Hagedorn, Peter H., Robert Persson, Erik D. Funder, Nanna Albæk, Sanna L. Diemer, Dennis J. Hansen, Marianne R. Møller, et al. 2018. "Locked Nucleic Acid: Modality, Diversity, and Drug Discovery." *Drug Discovery Today* 23 (1): 101–14. <https://doi.org/10.1016/j.drudis.2017.09.018>.
16. Matsui, Masayuki, and David R. Corey. 2017. "Non-Coding RNAs as Drug Targets." *Nature Reviews Drug Discovery* 16 (3): 167–79. <https://doi.org/10.1038/nrd.2016.117>.

References (continued)

17. Warner, Katherine Deigan, Christine E. Hajdin, and Kevin M. Weeks. 2018. "Principles for Targeting RNA with Drug-like Small Molecules." *Nature Reviews Drug Discovery* 17 (8): 547–58. <https://doi.org/10.1038/nrd.2018.93>.
18. Wang, Qiong, Yiqun Chen, Jaeyoung Park, Xiao Liu, Yifeng Hu, Tiexin Wang, Kevin McFarland, and Michael J. Betenbaugh. 2019. "Design and Production of Bispecific Antibodies." *Antibodies* 8 (3): 43. <https://doi.org/10.3390/antib8030043>.
19. Jensen, Karin J., Christian B. Moyer, and Kevin A. Janes. 2016. "Network Architecture Predisposes an Enzyme to Either Pharmacologic or Genetic Targeting." *Cell Systems* 2 (2): 112–21. <https://doi.org/10.1016/j.cels.2016.01.012>.
20. Suzuki, Masami, Chie Kato, and Atsuhiko Kato. 2015. "Therapeutic Antibodies: Their Mechanisms of Action and the Pathological Findings They Induce in Toxicity Studies." *Journal of Toxicologic Pathology* 28 (3): 133–39. <https://doi.org/10.1293/tox.2015-0031>.
21. Dammes, Niels, and Dan Peer. 2020. "Paving the Road for RNA Therapeutics." *Trends in Pharmacological Sciences* 41 (10): 755–75. <https://doi.org/10.1016/j.tips.2020.08.004>.
22. Levin, Arthur A. 2019. "Treating Disease at the RNA Level with Oligonucleotides." *New England Journal of Medicine* 380 (1): 57–70. <https://doi.org/10.1056/NEJMra1705346>.
23. Baranello, Giovanni, Basil T. Darras, John W. Day, Nicolas Deconinck, Andrea Klein, Riccardo Masson, Eugenio Mercuri, et al. 2021. "Risdiplam in Type 1 Spinal Muscular Atrophy." *New England Journal of Medicine* 384 (10): 915–23. <https://doi.org/10.1056/NEJMoa2009965>.
24. Ratni, Hasane, Martin Ebeling, John Baird, Stefanie Bendels, Johan Bylund, Karen S. Chen, Nora Denk, et al. 2018. "Discovery of Risdiplam, a Selective Survival of Motor Neuron-2 (SMN2) Gene Splicing Modifier for the Treatment of Spinal Muscular Atrophy (SMA)." *Journal of Medicinal Chemistry* 61 (15): 6501–17. <https://doi.org/10.1021/acs.jmedchem.8b00741>.
25. Sivaramakrishnan, Manaswini, Kathleen D. McCarthy, Sébastien Campagne, Sylwia Huber, Sonja Meier, Angélique Augustin, Tobias Heckel, et al. 2017. "Binding to SMN2 Pre-mRNA-Protein Complex Elicits Specificity for Small Molecule Splicing Modifiers." *Nature Communications* 8 (November): 1476. <https://doi.org/10.1038/s41467-017-01559-4>.
26. Singh, N. N., M. D. Howell, E. J. Androphy, and R. N. Singh. 2017. "How the Discovery of ISS-N1 Led to the First Medical Therapy for Spinal Muscular Atrophy." *Gene Therapy* 24 (9): 520–26. <https://doi.org/10.1038/gt.2017.34>.



References (continued)

27. Roberts, Thomas C., Robert Langer, and Matthew J. A. Wood. 2020. "Advances in Oligonucleotide Drug Delivery." *Nature Reviews Drug Discovery* 19 (10): 673–94. <https://doi.org/10.1038/s41573-020-0075-7>.
28. Tambuyzer, Erik, Benjamin Vandendriessche, Christopher P. Austin, Philip J. Brooks, Kristina Larsson, Katherine I. Miller Needleman, James Valentine, et al. 2020. "Therapies for Rare Diseases: Therapeutic Modalities, Progress and Challenges Ahead." *Nature Reviews Drug Discovery* 19 (2): 93–111. <https://doi.org/10.1038/s41573-019-0049-9>.
29. The Shape of Drugs to Come, Amgen, <https://www.amgenscience.com/features/the-shape-of-drugs-to-come/>
30. Citri, Ami, and Yosef Yarden. 2006. "EGF–ERBB Signalling: Towards the Systems Level." *Nature Reviews Molecular Cell Biology* 7 (7): 505–16. <https://doi.org/10.1038/nrm1962>.
31. SelleckChem tool compounds for the EGFR pathway, [https://www.selleckchem.com/EGFR\(HER\).html](https://www.selleckchem.com/EGFR(HER).html)
32. Zhang, M. May, Raman Bahal, Theodore P. Rasmussen, José E. Manautou, and Xiao-bo Zhong. 2021. "The Growth of SiRNA-Based Therapeutics: Updated Clinical Studies." *Biochemical Pharmacology*, January, 114432. <https://doi.org/10.1016/j.bcp.2021.114432>.
33. Wikipedia, RNA splicing, https://en.wikipedia.org/wiki/RNA_splicing
34. Wikipedia, RNA splicing, work by Agathman, used under CC-BY-3.0, https://commons.wikimedia.org/wiki/File:A_complex.jpg
35. Scotti, Marina M., and Maurice S. Swanson. 2016. "RNA Mis-Splicing in Disease." *Nature Reviews Genetics* 17 (1): 19–32. <https://doi.org/10.1038/nrg.2015.3>.
36. Jutzi, Daniel, Maureen V. Akinyi, Jonas Mechtersheimer, Mikko J. Frilander, and Marc-David Ruepp. 2018. "The Emerging Role of Minor Intron Splicing in Neurological Disorders." *Cell Stress* 2 (3): 40–54. <https://doi.org/10.15698/cst2018.03.126>.
37. Smith, C.I. Edvard, and Rula Zain. 2019. "Therapeutic Oligonucleotides: State of the Art." *Annual Review of Pharmacology and Toxicology* 59 (1): 605–30. <https://doi.org/10.1146/annurev-pharmtox-010818-021050>.
38. Fakhr, E., F. Zare, and L. Teimoori-Toolabi. 2016. "Precise and Efficient SiRNA Design: A Key Point in Competent Gene Silencing." *Cancer Gene Therapy* 23 (4): 73–82. <https://doi.org/10.1038/cgt.2016.4>.
39. Bennett, C. Frank, and Eric E. Swayze. 2010. "RNA Targeting Therapeutics: Molecular Mechanisms of Antisense Oligonucleotides as a Therapeutic Platform." *Annual Review of Pharmacology and Toxicology* 50 (1): 259–93. <https://doi.org/10.1146/annurev.pharmtox.010909.105654>.

Supplementary Information

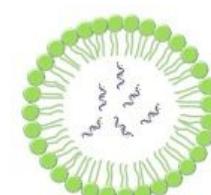
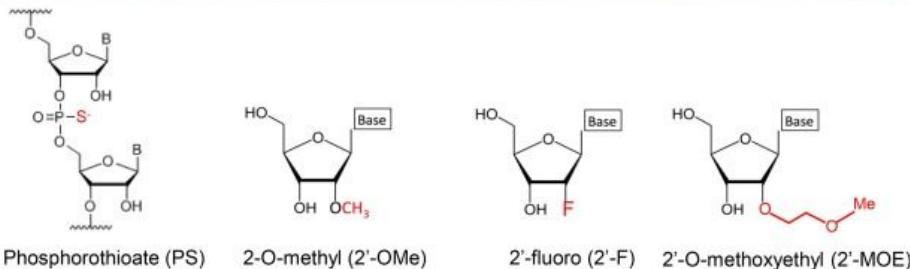
The growth of siRNA-based therapeutics

FDA-approved siRNA drugs	Patisiran	Givosiran	Lumasiran	
siRNA drugs in clinical trials	Vutrisiran	Nedosiran	Inclisiran	Fitusiran
	Teprasiran	Cosdosiran	Tivanisiran	
Drug	Alternative name	Company	Disease	Updated status
Patisiran	ONPATTRO	Alynlam	Hereditary transthyretin mediated amyloidosis	FDA approval in 10/08/2018 210922Orig1s000*
Givosiran	GIVLAARI	Alynlam	Acute hepatic porphyria	FDA approval in 11/20/2019 212194Orig1s000
Lumasiran	ALN-GO1	Alynlam	Primary hyperoxaluria type 1 (PH1)	FDA approval on 11/23/2020 214103Orig1s000
Vutrisiran	ALN-TTRsc02	Alynlam	Hereditary transthyretin mediated amyloidosis	Phase 3 trials ELIOS-A (NCT03759379)** HELIOS-B (NCT04153149)
Nedosiran	DCR-PHXC	Dicerna Alynlam	Primary hyperoxaluria	Phase 3 trial PHYOX 3 (NCT04042402)
Inclisiran	ALN-PCSSC	Alynlam Novartis	Hypercholesterolemia	Phase 3 trials ORION-9 (NCT03397121) ORION-10 (NCT03399370) ORION-11 (NCT03400800)
Fitusiran	ALN-AT3sc ALN-APC SAR439774	Alynlam Sanofi Genzyme	Hemophilia A and B	Phase 3 trials ATLAS-A/B (NCT03417245) ATLAS-INH (NCT03417102) ATLAS-PPX (NCT03549871) ATLAS-PEDS (NCT03974113) ATLAS-OLE (NCT03754790)
Teprasiran	AKII-5, DGF1, I-5NP, QPI-1002	Quark Novartis	Acute kidney injury Delayed graft function	Phase 3 trial ReGIFT (NCT02610296)
Cosdosiran	QPI-1007	Quark	Non-arteritic anterior ischemic optic neuropathy (NAION)	Phase 2/3 trial NCT02341560
Tivanisiran	SYL-1001	Sylentis	Dry eyes Ocular pain	Phase 3 trial HELIX (NCT03108664)

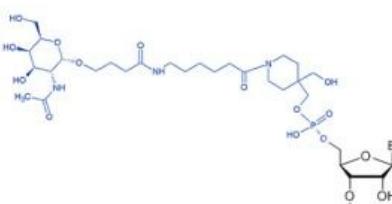
* FDA application number.

** ClinicalTrials.gov identifier number at <https://clinicaltrials.gov/ct2>

Drug	Backbone	Chemical modifications			Delivery platform
		PS	2'-OMe	2'-F	
Patisiran	-	+ (11)	-	-	LNP
Givosiran	+ (6)	+ (28)	+ (16)	-	GalNAc
Lumasiran	+ (6)	+ (34)	+ (10)	-	GalNAc
Vutrisiran	+ (6)	+ (35)	+ (9)	-	GalNAc
Nedosiran	+ (6)	+ (35)	+ (19)	-	GalNAc
Inclisiran	+ (6)	+ (32)	+ (11)	+ (1)	GalNAc
Fitusiran	+ (6)	+ (23)	+ (21)	-	GalNAc
Teprasiran	-	+ (19)	-	-	None
Cosdosiran	-	+ (9)	-	-	None
Tivanisiran	-	-	-	-	None



Lipid nanoparticle (LNP)



N-acetylgalactosamine (GalNAc)